GROWTH-REGULATING SUBSTANCES AS HERBICIDES

E. J. KRAUS AND J. W. MITCHELL[1]

Introduction

Soon after the discovery of the possible use of indoleacetic acid as a means of inducing root formation by some plants, it became obvious that there was a close relationship between the quantity and concentration of the acid used and the degree and type of response made by treated plants. It was clear, also, that the period of time over which a plant was exposed to the growth-regulator was important. It was necessary, therefore, to observe a critical quantity–concentration-time factor in the treatment of cuttings, seeds, or entire plants for any desired result (**4, 10, 16, 17**). Other significant observations were that various species, varieties, and even individual plants showed a marked differential in the degree of their responses to different substances in varying concentration under the same environment and that the responses were markedly influenced by varying environmental conditions. It was found that the chemical composition and the degree of maturity of the plant treated had much to do with its qualitative and quantitative responses after treatment. As more potent growth-regulators were discovered and studied, and as attempts were made to adapt them to economic use, the importance of all these facts became more strikingly clear and obvious. Many of the earlier, and also of the current, investigations have been concerned with the testing of plant behavior in these respects. Only a few of many earlier references are given to indicate these points (**10, 11, 12**).

Briefly summarized, it had been recognized almost from the inception of the attempted economic use of synthetic plant hormones or growth-regulating substances that there was a fairly wide range in the degree to which various species or even individual plants would respond. It was necessary to determine experimentally the range within which these compounds could be applied to plants without toxic injury (**4, 10, 17**). For many years, in the United States at least, although apparently less so in Great Britain (**14, 17**), few attempts were made to utilize growth-regulators as plant toxicants. Much greater attention was given to the potential capacity of growth-regulators to suppress bud development or to stimulate or increase plant growth, and other miscellaneous effects, especially by experimentalists in the applied fields. That there has been a marked change in viewpoint is well shown by the very large numbers of recent reports on the use of growth-regulators as herbicides. It is greatly to be hoped that a reasonable balance in viewpoint will prevail and that studies on the full range of the effects which these compounds may produce in and upon plants will receive the adequate attention such studies well merit.

When, prior to 1941, items of national defense became of increasingly greater concern, it was obvious to us, because of the closeness with which we had worked on growth-regulating substances for several years, that far more thought

[1] Senior Physiologist, Bureau of Plant Industry, Soils and Agricultural Engineering, Beltsville, Md.

and attention should be given to any defensive measures which could be employed if advantage were to be taken of the toxic properties of growth-regulating substances for the destruction of crops or the limitation of crop production. Obviously, such studies could, at the same time, demonstrate any value which such compounds might possess as offensive tools. Special advantage could be taken of the differential sensitivity of plants to growth-regulators by using the latter as differential herbicides and in other ways in agricultural practice. What we suggested was that the experience and knowledge already at hand, together with that from additional investigations concerning plant responses to growth-regulators, be used more nearly completely than had been the case. Our suggestions were brought to the attention of certain groups of individuals which had been organized to concern themselves with problems of national defense. The idea was also presented to representatives of the United States Army. First at the University of Chicago and then under sponsorship of a committee of the National Academy of Sciences, investigations were begun and results reported from time to time. The work eventually became a part of an established program on biological warfare. All details concerning this work, as well as all results obtained, were promptly placed under restriction. Some of the pertinent facts concerning the program on biological warfare and its implications were presented by Mr. G. W. MERCK (**3, 9**) at the Westinghouse Centennial Forum in May, 1946. These need not be reviewed here. Some of the results of work carried out as part of the activities of investigators in the Special Projects Division, Chemical Warfare Service, at Camp Detrick, Frederick, Maryland, have appeared in a series of eighteen papers in the BOTANICAL GAZETTE for June, 1946 (**107**:476–632), and elsewhere (**2**). This program dealing with the use of growth-regulators as herbicides was well advanced before it was known to us that important developments in a similar field also took place in Great Britain (**15, 18**).

As has been pointed out by NORMAN (**13**), our earlier data have been withheld from publication up to now in conformity with security regulations. It still seems worth while to release them because they present certain results concerning the reactions of a number of species and varieties of plants apparently not yet recorded elsewhere. The points concerning the recovery and subsequent behavior of plants injured in varying degrees by the several growth-regulators, the resultant, obvious stimulating effects, as indicated by the increased intensity of the green color, larger size, and greater longevity of some plants, as well as various other details, deserve much further investigation.

In our earlier experiments growth-regulating substances then in common use were applied as sprays, dusts, and vapors and by other methods. By request, experiments with some of the better-known inorganic and organic herbicides, both differential and nondifferential, were carried out and the results reported. Among these materials three plant growth-regulators appeared to have some importance. In the case of bean plants, phenylacetic acid applied at the rate of 1 p.p.m. in water to soil decreased growth 75%. As an emulsion sprayed on the foliage, it decreased growth 10% compared with controls. Naphthaleneacetamide applied at the rate of 1 part in 10 million in water to soil decreased growth 70–100%, and the same concentration applied as an emul-

sion spray to the foliage reduced growth 25%. These results were somewhat similar to those already reported by other investigators (**5, 6, 8, 14, 17**). A 20% emulsion spray of α-naphthaleneacetic acid killed 70% of the treated plants. Results with other compounds were similar to those already frequently reported in the literature as dwarfing growth and, generally, decreasing yields of fruits or grains (**10, 14, 17**).

rophenoxyacetic acid, and 2,3,5-triiodobenzoic acid (fig. 1). A trial with most encouraging results was made in January, 1943, in the greenhouses at the University of Chicago. More extensive investigations were begun as soon as possible at the United States Plant Industry Station, Beltsville, Maryland. Data on some of the earlier trials are given herewith. In the descriptions below, trials are distinguished by letter for convenience in

FIG. 1.—Black Valentine bean plants. *Left*, untreated. *Right*, sprayed with a water emulsion containing 0.5% of 2,3,5-triiodobenzoic acid and 1% of Emulphor AG; 3 weeks after treatment.

Early in 1942 ZIMMERMAN (**1, 19, 20**) called attention to the high potency of certain substituted phenoxyacetic acid and benzoic acid growth-regulators. It occurred to us that these might well be of great value in connection with the experiments we had under way. Accordingly, samples of some of these materials were requested from Dr. ZIMMERMAN, who promptly furnished us with material of 2,4-dichlorophenoxyacetic acid, 2,4,5-trichlorophenoxyacetic acid, 4-chlo-

reading; the letters do not indicate the number or the sequence of the experiments as conducted.

Experimentation

TRIAL A

Young kidney bean plants were grown in rich soil in pots placed on well-lighted greenhouse benches at the Plant Industry Station. Two plants were left in each pot. At the time of treatment the first

trifoliate leaf was just beginning to expand. The plants were decapitated just below the node at which such a leaf was borne. Each growth-regulator used was added to lanolin, making a 2% mixture, and was applied in a thin film to the cut surface on March 22, 1943. The following notes were made on March 30, 1943.

Tryptophane.—Growth about same as in previous experiments (**7**); somewhat less than when glutathione was added to mixture.

Tryptophane (2%)+glutathione (2%).—Considerable growth made by entire plant; more than controls. Tumors formed show more growth than controls. Tumors irregular, large; many large galls formed at points where shoots had been removed. Galls about same size as tryptophane only but somewhat fewer in number. May be no correlation with composition of mixture applied.

α-Naphthyl methyl acetate.—Plants very responsive; tissues killed at surface and much swollen below. Many root primordia. Tumors decidedly ridged in appearance; resemble those from application of naphthaleneacetic acid more than any others.

4-Chlorophenoxyacetic acid.—Stem dead to primary leaves, which have turned yellow. Some injury below primary leaves. A few plants show tumors with dead stem above them. Mixture too concentrated.

2,4,5-Trichlorophenoxyacetic acid.—Results similar to application of 4-chlorophenoxyacetic acid. More plants show severe injury, especially farther down the stem. Leaves yellowed; some plants dead.

2,4-Dichlorophenoxyacetic acid.—Less injury than 4-chlorophenoxyacetic acid or 2,4,5-trichlorophenoxyacetic acid. Tumors flatter when formed—more thickening of stems at greater distance. Substance apparently travels farther through plant, but plants are too severely injured to continue long development. Try weaker mixture.

α-Naphthaleneacetic acid.—Large tumors as usual, about like those resulting from application of α-naphthyl methyl acetate but appreciably smaller; not so flat; fewer roots, about same amount of injury.

Trial B

Another set of lanolin mixtures was applied on April 9, 1943, at Beltsville. The bean plants were grown in pots of rich loam in a well-lighted greenhouse, two plants per pot. They were more vigorous than the first lot treated on March 22, 1943. The decapitation method was used; the trifoliate leaf was expanding at the time of treatment.

Notes were made and specimens collected on April 26, 1943. The concentration of each growth substance used is given for each particular treatment.

Tryptophane (2%).—From the same bottle used in trial A. Results about the same as in first experiment, but many fewer galls developed where shoots had been removed. Since there was more space between plants, there may have been less moisture in the air around the plants. Plants grew more rapidly than in trial A, but tumors were no larger.

Tryptophane (2%)+glutathione (2%).—This mixture was the same as used in trial A, and results are similar. Addition of glutathione to the tryptophane mixture gives definite stimulation in early stages of growth but does not result in a finally larger tumor. Tumors on sides of stems somewhat larger than with tryptophane only. This may be the result of more light and a difference in moisture content of the atmosphere.

4-Chlorophenoxyacetic acid (1%).—A thin film was applied on cut surface, but some ran down the side when it melted in sun. Material from same vial as in trial A, but diluted with an equal volume of lanolin. Treated stems usually killed halfway to primary leaves, where they then enlarged to form large oval tumors with prominent ridges and some root primordia. Axillary shoots 1–5 inches long; some normal, but most of them have tightly rolled, very narrow leaflets, curled at the tip. Leaves striped dark- and light-green like those on plants showing symptoms of mosaic. Stimulus apparently travels for long distances in the plants. Primary leaves yellow. Axillary stems not much distorted. (Fig. 2*B*, *D*, *E*.)

2,4-Dichlorophenoxyacetic acid (1%).—Results similar to applications of 4-chlorophenoxyacetic acid. Tumors flatter; more root primordia, about 2 mm. below cut surface. Centers of tumors dead. Lateral shoots more deformed than with 4-chlorophenoxyacetic acid, often thickened and incurved like crab claws. This effect is much more pronounced than that fol-

lowing application of any other chlorophenoxyacetic acid compound. Some shoots are nearly normal if tumor is small, but many show some of the upper leaves with mosaic-like symptoms, the green areas greener than normal. This compound translocated for the longest distances of any tried and apparently is most toxic of any applied. (Fig. 2*A*.)

2,4,5-Trichlorophenoxyacetic acid (1%).—Growth responses similar to the two former chlorophenoxyacetic acid compounds, but tumors larger and flatter, flanged, the flanges with roots. Center of tumors dead. There are some root primordia on the stem above primary leaves, but tumor is distinctive. Some axillary shoots appear nearly normal and have a few flower buds; some have strong mosaic-like appearance; a few with crab claws at tips of shoots. Apparently translocated less distance and has less destructive action than 4-chlorophenoxyacetic acid or 2,4-dichlorophenoxyacetic acid. (Fig. 2*C*.)

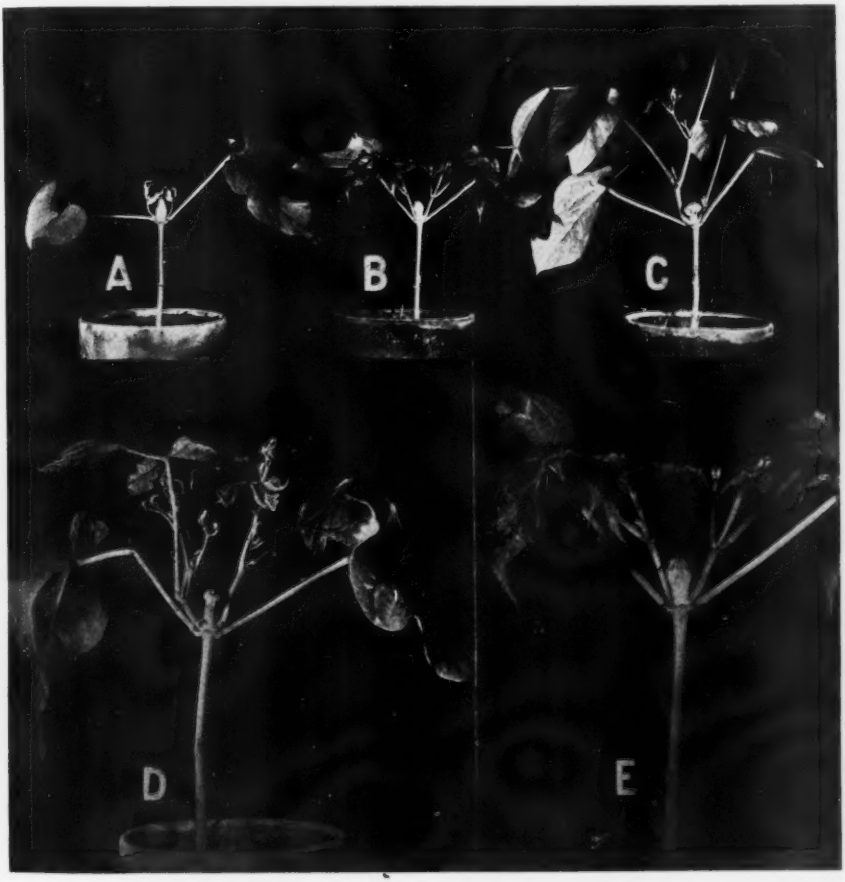

FIG. 2.—Response of Red Kidney beans to terminal applications of 1% mixtures of three different chlorophenoxyacetic acids in lanolin. *A*, 2,4-dichlorophenoxyacetic acid; *B*, 4-chlorophenoxyacetic acid; *C*, 2,4,5-trichlorophenoxyacetic acid, showing large flanged tumors; *D*, 4-chlorophenoxyacetic acid; *E*, closer view of *B*. *B*, *D*, and *E* show what is referred to in text as effects resembling "mosaic-like symptoms." Treated April 9, 1943; photographed April 26, 1943.

TRIAL C

A third set of mixtures was applied in the greenhouses at Beltsville. As in the other trials, the beans were thinned to two per pot. The plants were more vigorous and larger than in either of the other two trials. The trifoliate leaves were just spreading out; on a few plants they still were not folded down. The weather was warmer, and there was more sun than for previous lots of plants. Fewer of the plants were slender. The decapitation method was used, and lanolin mixtures at the concentrations indicated were applied to the cut surfaces on May 11, 1943. The following notes were made on May 26, 1943.

N-(β-naphthyl)-glycine (1.5%).—Very slight response; no killing; large axillary shoots apparently not injured—many with large buds and few flowers. About like lanolin-treated controls.

Benzothiazyl thioglycolic acid (1.5%).—Very little response, more than to foregoing but not so much as made by some of controls, which have large phloem galls. Shoots not injured; buds and some flowers about as in controls.

β-Naphthoxy α-propionic acid (1.5%).—Extremely large, knobby tumors dead at center, flanged, and with many root primordia. A few plants grew very little. Not much apparent effect below tumors. Shoots large, similar to controls. A few petioles and blades of the primary leaves have tumors with roots. Translocation questionable; tumors on leaves possibly resulted from contact with tip of stem while being treated.

α-Naphthyl methyl acetate (1.5%).—Extremely large tumors, biggest of any in this experiment. They resemble those resulting from application of β-naphthoxy α-propionic acid as indicated above but are larger, less tuberculated, and with fewer roots. Some flanges on stem below tumor; a few root primordia ¾ inch from treated surface. Axillary shoots dwarfed somewhat (often only one large one), shorter than controls. Plants smaller but about same stage of bloom as controls.

Phenazine (1.5%).—Little or no tumor response, but tissues apparently killed near surface. No tumors, as is true for controls. Axillary shoots about same as those of controls. Flower buds and few flowers present.

Of the same lot of plants as those used for the terminal applications to decapitated internodes, some were thinned to two plants per pot and arranged in rows on the greenhouse benches. A thin thread of lanolin–growth-substance mixture was wound around the second internode just below the trifoliate leaf. The same mixtures and concentrations as were used in making the terminal applications were employed. Growth substances were applied on May 11, and notes were taken on May 25, 1943.

N-(β-naphthyl)-glycine (1.5%).—Stems yellowed, some showing swelling although scarcely visible. No apparent injury or stimulation.

Benzothiazyl thioglycolic acid (1.5%).—Apparently no response other than yellowing of stems at point of application and slight spindle-like swelling. Plants resemble controls in all respects.

β-Naphthoxy α-propionic acid (1.5%).—Many large spindle-shaped tumors, ¼–1 inch long, flanged with many root primordia. Some tips died soon after treatment. Such plants are sometimes dead to the primary leaves. Often they have big terminal tumors resembling those following terminal applications, but with dead stem tip at center. Slight dwarfing effect; otherwise like controls.

α-Naphthyl methyl acetate (1.5%).—Very great response, long spindle-shaped tumors, sometimes nearly full length of an internode. Larger portion of tumor is below line of treatment than above it. Some terminals dead, others vigorous, especially if only small tumor is present. Tumors rough, flanged, many root primordia and rather spongy. Little effect on the primary leaves (many are shed) or on portion of plant below them. If big tumor is present and stem tip is dead, strong axillary shoots are present similar to controls. Response somewhat resembles that to naphthaleneacetic acid except it is greater.

Phenazine (1.5%).—No visible effect.

4-Chlorophenoxyacetic acid (1%).—Very large long galls, sometimes extending below primary leaves. Tips of stems frequently killed or much swollen, resembling small potatoes above trifoliate leaf which often shows effects resem-

bling symptoms of mosaic. Axillary shoots much as in terminal treatment except that mosaic-like appearance is more pronounced and leaves are curled, especially smaller leaves, which often are yellow.

2,4,5-Trichlorophenoxyacetic acid (*1%*).—Very similar to responses to 4-chlorophenoxyacetic acid except more pronounced; effects show far greater distance down the stem. These effects seem greater than following terminal application of mixture. Some shoots have flower buds and flowers. Leaves are large as in controls; green areas of leaves which show mosaic-like symptoms seem darker. Some of the "unaffected" leaves also appear darker than those of controls. Whole plant is more bushy than controls.

2,4-Dichlorophenoxyacetic acid (*1%*).—In nearly every respect similar to responses following application of 2,4,5-trichlorophenoxyacetic acid, but plants seem more branched and bushy. They are much dwarfed compared with controls. Some have died completely, starting from the tops down. Earlier they showed root primordia and swelling below primary leaves. On some nearly dead plants, a leaf or two still appears fairly green or partially yellow. Some plants have many small shoots at cotyledonary nodes. These shoots have many leaves and clusters of buds. Most of the live plants are twiggy in appearance; others have large green shoots. Responses very variable. This may be the result of the degree of contact made in application, or owing to individual response of plant treated.

Controls (*May 26, 1943*).—Plants which had been decapitated are now 16–20 inches tall. Many of them have flower buds, flowers, and young fruits. Many of the shoots in the axils of primary leaves are as large as is the main axis below them. The primary leaves of many plants are yellowed or abscised. Plants which were treated laterally with lanolin only are generally similar to plants treated terminally with lanolin only, except that they are somewhat taller.

Trial D

During the period in which some of the foregoing experiments were in progress at Beltsville an experiment dealing with the spraying of rice was begun and carried out at the University of Chicago. Two varieties of rice, Blue Rose and Fortuna, were grown in large concrete tanks in a greenhouse. On May 14, 1943, the plants were about 12–14 inches tall, vigorous, but not yet showing heads. The water in the tanks was generally maintained 1½–2 inches in depth and developed a very slight surface scum. Three of the tanks were 84×36 inches; the fourth was 76×36 inches. The water level in the tanks was about ¾ inch deep when they were sprayed. One tank was sprayed with 100 mg. of 4-chlorophenoxyacetic acid, another with the same quantity of 2,4-dichlorophenoxyacetic acid, and a third with the same quantity of 2,4,5-trichlorophenoxyacetic acid. Before application each compound was first mixed with 5 gm. of melted lanolin and then made up to 100 cc. as an emulsion with a 5% solution of laundry soap.

These emulsions were prepared by dissolving the required amount of growth-regulator in melted lanolin and then pouring this melted mixture into the hot soap solution while being stirred vigorously in a Waring blendor. When well mixed, the emulsion was diluted to any desired volume. Because of the alkalinity of the soap used in preparing the emulsion, it is probable that salts of the several acids were formed. The diluted emulsion was then sprinkled over the surface of the water in the respective tanks. The emulsion spread slowly over the water, which became turbid. It was then stirred slightly with a stick to secure uniform spread of the emulsion over the surface and throughout the water. The material tended slightly to collect around the stems of the plants, forming a thin film on their surface, ½ inch or more above the water level.

On May 16, 1943, it was noted that there was not much change in appearance of the treated plants except that all showed more yellowing of the tips of the

leaves than did the controls. No bending or other changes were observed except that the sheathing leaves at the base of the plants appeared slightly water-soaked somewhat higher above the water level than did those of the controls. These basal sheaths were dead and black in all plants. There was some apparent injury, but it was not nearly so pronounced as is usually shown by beans after the same length of time.

The next opportunity to make observations on these plants was on June 25, 1943. The unsprayed rice was green, and many of the plants showed the beginnings of heads or heads partially emerged. In the tank sprayed with 4-chlorophenoxyacetic acid emulsion, most of the rice plants had the leaves dead near the surface of the water. Some plants were fairly green but none had heads. Many were dead. Nearly all the plants sprayed with the 2,4,5-trichlorophenoxyacetic acid emulsion were dead and dried up except those near the corners of the tank. The appearance of the plants sprayed with the 2,4-dichlorophenoxyacetic emulsion was about the same as of those sprayed with 2,4,5-trichlorophenoxyacetic acid emulsion. This tank was in the northwest corner of the greenhouse and deeper than the others. The water in it might have been deeper when sprayed, and there was more distance from the water level to the top of the tank. 2,4-Dichlorophenoxyacetic acid was about as potent as 2,4,5-trichlorophenoxyacetic acid emulsion, but its application resulted in the killing of more plants than 4-chlorophenoxyacetic acid emulsion.

TRIAL E

Another trial was begun to test further the relative toxicity of certain substances and especially the degree to which they or their effects were translocated within the plant to which they had been applied. Red Kidney bean plants were used in the greenhouses at the University of Chicago. On September 26, 1943, when the substances were applied, the plants were vigorous but somewhat spindling because of the prevailing low light intensity. On most of the plants the first trifoliate leaves were expanding. On the others this leaf was fairly well expanded, and the internode above it was 2–3 inches long and rather slender. The plants were grown in pots of soil, two to each pot, and were spread out in rows on greenhouse benches. Each of the growth-regulating substances was dissolved in, or thoroughly mixed with, melted lanolin and applied at a 1.5% concentration. Applications were made in various ways: the usual terminal application after decapitation, thin bands applied laterally to uninjured stems, or in other ways as specifically indicated in each test. The appended notes were taken on October 8, 1943.

The chlorophenoxyacetic acid compounds used in this experiment were prepared by Dr. E. D. WITMAN, Ohio State University Research Foundation. Some of the other compounds used are of the same lot of materials mentioned in previous trials.

4-Chlorophenoxyacetic acid.—Terminal. Large tumors, many extending to soil line. Some plants have no lateral shoots; if present, these are small, deformed, and show mosaic-like symptoms. Some plants are dead and yellow.

Same.—Lateral. Long tumors; some terminating at node, others extend nearly to soil. Trifoliate leaves much distorted; tips of stems above leaves often thick, swollen, and yellow; some of them dead. Some whole plants dead with leaves yellow but not so many as in terminal treatment.

2,4-Dichlorophenoxyacetic acid.—Terminal. Very long, large tumors. Many plants killed, including roots; many show mosaic-like appear-

ance of lateral shoots similar to 4-chlorophenoxyacetic acid but more intense. Many roots on stems. Some plants very much thickened, yellow, dead or dying.

Same.—Lateral. Similar to terminal treatment. More plants dead. Tips of most plants above ring of treatment dead; some much curled, twisted, or tuber-like. Many tumors extend to soil surface; plants curled over. *Stronger killing action than 4-chlorophenoxyacetic acid.*

2,4,5-Trichlorophenoxyacetic acid.—Terminal. Very similar to 2,4-dichlorophenoxyacetic acid in all respects except that effects are less pronounced.

Same.—Lateral. Effects very similar to those of 2,4-dichlorophenoxyacetic acid applied laterally. Some plants seem to be more injured, others less so. Effects do not seem to extend so far throughout plant as in case of 2,4-dichlorophenoxyacetic acid.

2,4,6-Trichlorophenoxyacetic acid.—Terminal. Almost no tumor formed. Yellowing near point of application. Very slight growth at surface.

Same.—Lateral. Virtually no response. Very different from 2,4-dichlorophenoxyacetic or 2,4,5-trichlorophenoxyacetic acid. (NOTE.—Repeat using new preparation or mixture.)

β-Naphthoxy α-propionic acid.—Terminal. Terminal tumors similar to those in previous experiments performed at Beltsville but not so large or extensive. Effects more localized. May be that plants are less active now; they are more spindling.

Same.—Lateral. Small spindle-like tumors very similar to those produced when naphthaleneacetic acid was applied, but somewhat more rough. Effects localized. Tops above treatment somewhat dwarfed, but no effects comparable in degree with chlorophenoxyacetic acid compounds.

α-Naphthyl methyl acetate.—Terminal. Medium-sized, spreading tumors recurved at edges; smaller than at Beltsville in spring, more localized, much less injury. Axillary shoots large.

Same.—Lateral. Similar to tumors produced by application of β-naphthoxy α-propionic acid but smaller and more localized. Not much injured, but plants are smaller than controls. Resembles effects of naphthaleneacetic acid, but tumors are somewhat smaller.

2,4-Dichlorophenoxyacetic acid.—Not decapitated. Applied as small pellet on vein at middle of primary leaf. Vein enlarged; yellow spot only on leaf, but effect very marked on axillary shoots and tips of plant which are either mosaic-like or thickened and swollen. Trifoliate leaves often curled, dwarfed, mosaic-like. Petioles of primary leaves show almost no swelling. Some leaves dead. Effects shown by axillary shoots are as great as when substance is applied terminally.

2,4,5-Trichlorophenoxyacetic acid.—Method of application same as above. Effects similar to those from 2,4-dichlorophenoxyacetic acid but average slightly less. Effects of conduction for long distances very marked. More mosaic-like in appearance with less swelling of tips of stems and young leaves than in case of 2,4-dichlorophenoxyacetic acid. (NOTE.—Repeat on younger and older plants.)

2,4,6-Trichlorophenoxyacetic acid.—Method of treatment same as for 2,4-dichlorophenoxyacetic acid above. Apparently no effect except small yellow spot. (NOTE.—See terminal and lateral applications. Repeat using new mixture.)

2,4-Dichlorophenoxyacetic acid.—Applied to tip of middle leaflet of partially expanded first trifoliate leaf above long, slender second internode. Leaflet sometimes killed. Much less effective than when applied on vein, but tip of stem above treated leaf often tuberous, sometimes dead. Other two leaflets of leaf often deformed. Sometimes no effect if tip of treated leaflet has died. Very little effect shown by axillary shoots.

2,4,5-Trichlorophenoxyacetic acid.—Applied as in 2,4-dichlorophenoxyacetic acid treatment. Effects similar to 2,4-dichlorophenoxyacetic acid. Tips of more leaflets killed; such plants often show no injury to tip of stem. Apparently less conduction following this method of application than when substance is applied on vein. Some leaves appear greener than those of controls.

2,4,6-Trichlorophenoxyacetic acid.—Method of treatment same. Plants are at east end of greenhouse and received more light. These plants are somewhat taller and more advanced than the others. No resultant effects after treatment except some tips of leaflets are yellow and a few dwarfed. Some of the primary leaves are yellow and have fallen. Plants resemble controls in all respects.

Indoleacetic acid.—Terminal. Tumors similar to those usually produced following application of this compound but are somewhat smaller than in some previous experiments.

α-Naphthaleneacetic acid.—Terminal. Responses similar to those usually following treatment with this compound, but tumors some-

what smaller and with fewer root primordia. Some tumors have died; in such cases the stem is dead to the primary leaves. Large axillary shoots.

Same.—Lateral. Similar to previous comparable experiments, except tumors are shorter and with not many tubercles or flanges. Tops above region of application dwarfed but not killed; few root primordia.

Same.—On midvein of primary leaf. Yellow spot on some leaves; others have raised area at point of application. Some leaves have medium-sized tumor with root primordia. Vein sometimes yellow. Leaf shows slight injury but may be strongly curled. Not much effect on axillary shoot. None of treated leaves abscised. They are greener in color than controls.

Controls (lanolin only).—Not decapitated. Many are vinelike, 24 inches tall, slender, with flower buds or flowers. More slender than plants which received more sunshine. Primary leaves yellowing, some fallen, others green. Plants at east end of greenhouse are larger than those near door at west end. Some of them with one or two flowers and some buds.

Same.—Decapitated. Large axillary shoots, a few with flower buds. Most of decapitated internodes shriveled to near primary leaves; a few have phloem tumors at their tips. Most of axillary shoots are not vinelike except some which are near door. These latter do not have so many buds as at east end of greenhouse where plants are more bushy.

NOTE.—Repeat experiments using chlorophenoxyacetic compounds. These are by far the most promising as herbicides and are conducted long distances in plants; other compounds result in more localized response. Try further emulsion sprays on leaves.

The lack of response to applications of 2,4,6-trichlorophenoxyacetic acid seemed surprising when compared with the other chlorophenoxyacetic acid compounds, but similar results were obtained with repeated trials. ZIMMERMAN and HITCHCOCK (20) have also reported like results.

TRIAL F

In January, 1944, another set of experiments was initiated at the University of Chicago.[2] Their immediate purpose was to determine, first, the most active of the compounds already tested and, second, to ascertain their effectiveness in decreasing the over-all growth and productivity of several types of plants when applied in relatively small amounts.

Barley, oats, corn, wheat, kidney bean, buckwheat, radish, rape, red clover, and sugar-beet plants were grown from seed in soil contained in flats which measured approximately 15×24 inches. In addition, two varieties of rice (Blue Rose and Fortuna) were grown in concrete tanks measuring approximately 36×72 inches and filled with soil to a depth of 6 inches. Seeds of the different plants were sown during January and early February. In the case of sugar beet and kidney bean two successive plantings were made so as to obtain plants of different ages. One sowing was made on January 16, 1944; the other, on February 3, 1944. The bean plants referred to as "old" in the photographs were sown on the former date; those as "young," on the latter. After the rice had germinated, a sufficient quantity of water was added to each tank to cover the soil to a depth of approximately 2 inches.

The plants were grown for a period of from 2 to 6 weeks before treatment, during which time relatively cloudy weather prevailed. At the time of treatment all plants, except early plantings of beets and beans, were yet immature and relatively succulent.

The growth-regulating compounds used included the most active of those known at the time these experiments were initiated, namely:

2,4-Dichlorophenoxyacetic acid
Penta-chlorophenoxyacetic acid

[2] Dr. CHARLES L. HAMNER, at the time Assistant Plant Physiologist in the Bureau of Plant Industry, Soils and Agricultural Engineering, assisted in the application of some of the compounds in this experiment and understood its objectives.

2-Chlorophenoxyacetic acid
4-Chlorophenoxyacetic acid
2,4,5-Trichlorophenoxyacetic acid
2,3,5-Triiodobenzoic acid
α-Naphthaleneacetic acid
α-Naphthyl methyl acetate

Data as to methods of treatment are given in table 1. The numbers used there are the same as those given in some of the data presented here and as shown on the small labels in the photographs (figs. 3–34). These labels also indicate the concentration of the growth-regulator used in each instance. All photographs were taken 10 days after treatment.

TABLE 1

GROWTH SUBSTANCES USED, METHOD OF APPLICATION, AND CONCENTRATIONS USED, FEBRUARY 29, 1944

Trial number and substance used	Method of application	Concentration
1. 2,4-Dichlorophenoxyacetic acid	Aerosol	12.6% in oil
2. 4-Chlorophenoxyacetic acid	Aerosol	6.3% in oil
3. Penta-chlorophenoxyacetic acid	Aerosol	1.26% in oil
4. α-Naphthaleneacetic acid	Lanolin emulsion	1%, 0.5%, 0.25%, 0.13%
5. 2,3,5-Triiodobenzoic acid	Lanolin emulsion	1%, 0.5%, 0.25%, 0.13%
6. 2,4-Dichlorophenoxyacetic acid	Lanolin emulsion	1%, 0.5%, 0.25%, 0.13%
7. 4-Chlorophenoxyacetic acid	Lanolin emulsion	1%, 0.5%, 0.25%, 0.13%
8. 2-Chlorophenoxyacetic acid	Lanolin emulsion	1%, 0.5%, 0.25%, 0.13%
9. 2,4,5-Trichlorophenoxyacetic acid	Lanolin emulsion	1%, 0.5%, 0.25%, 0.13%
10. 2,4-Dichlorophenoxyacetic acid	Oil emulsion S.A.E. 40	1%, 0.5%, 0.25%, 0.13%
11. 2,4-Dichlorophenoxyacetic acid	Oil emulsion S.A.E. 10	1%, 0.5%, 0.25%, 0.13%
1. α-Naphthaleneacetic acid	Dust	1 part to 500 talc
2. α-Naphthaleneacetic acid	Dust	1 part to 5000 talc
3. α-Naphthyl methyl acetate	Dust	1 part to 500 talc
4. α-Naphthyl methyl acetate	Dust	1 part to 5000 talc
5. Penta-chlorophenoxyacetic acid	Dust	1 part to 500 talc
6. Penta-chlorophenoxyacetic acid	Dust	1 part to 5000 talc
7. 2,4-Dichlorophenoxyacetic acid	Dust	1 part to 500 talc
8. 2,4-Dichlorophenoxyacetic acid	Dust	1 part to 5000 talc

The 1% lanolin emulsions were prepared as follows. One gram of growth substance dissolved in 5 ml. of ethyl alcohol was added to 10 gm. of melted lanolin. This mixture was then poured into 100 ml. of hot 5% solution of laundry soap and thoroughly mixed in a Waring blendor. For the lower concentrations dilutions were made with a similar soap solution. Since the soap was strongly alkaline, it is probable that salts of the various growth substances were formed in the emulsions. This fact, and also the fact that there was little sunshine during the period of growth of the plants previous to treatment and for more than 10 days after treatment, may have resulted in less response on the part of some of the plants than might have been true in sunny weather. The emulsions were applied to the plants by means of hand sprayers at the rate of approximately 25 ml. per flat of plants (surface area approximately 2.5 sq. ft.).

Light- and heavy-oil emulsions were prepared in a manner similar to the lanolin emulsions except that 40 ml. (approximately 10 gm.) of Valvoline motor oil (S.A.E. 40 or S.A.E. 10) were substituted for the lanolin. The oil emulsions were applied in a manner similar to the lanolin emulsions.

FIG. 3.—Blue Rose rice. *Above*, untreated plants 38 days after emergence. *Below*, comparable plants 10 days after spraying with an oil (S.A.E. 40) emulsion containing 1% of 2,4-dichlorophenoxyacetic acid, at the rate of 10 ml./sq. in. of water surface. Tank 36×72 inches. Water in tank ½ inch deep at time of spraying.

FIG. 4.—Barley 44 days old when treated. *Top*, untreated. *Center*, treated with aerosol containing 2,4-dichlorophenoxyacetic acid (*left*) and with 1% lanolin emulsion of the acid (*right*). *Bottom*, treated with 1% lanolin emulsions of 4-chlorophenoxyacetic acid (*left*) and 2,4,5-trichlorophenoxyacetic acid (*right*).

FIG. 5.—*Top*, wheat 44 days old when treated. *Left*, untreated. *Right*, treated with aerosol containing 2,4-dichlorophenoxyacetic acid. *Bottom*, oat plants 44 days old when treated. *Left*, untreated. *Right*, sprayed with lanolin emulsion containing 2,4,5-trichlorophenoxyacetic acid.

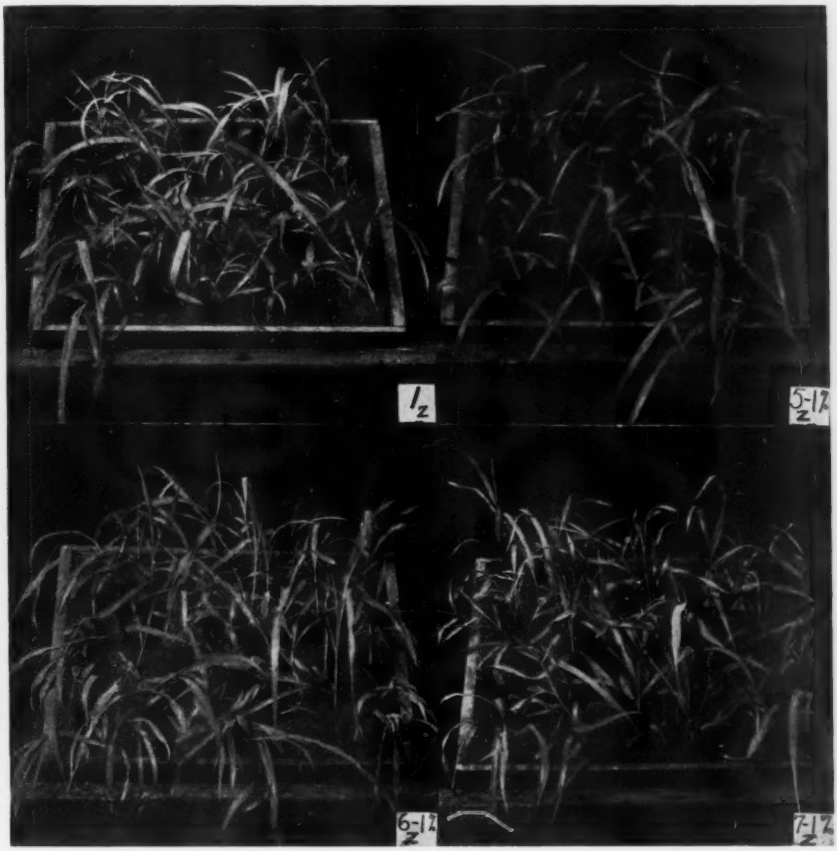

FIG. 6.—Corn plants sprayed with aerosol containing 2,4-dichlorophenoxyacetic acid (*top, left*) and with lanolin emulsions containing 2,3,5-triiodobenzoic acid (*top, right*), 2,4-dichlorophenoxyacetic acid (*bottom, left*), and 4-chlorophenoxyacetic acid (*bottom, right*).

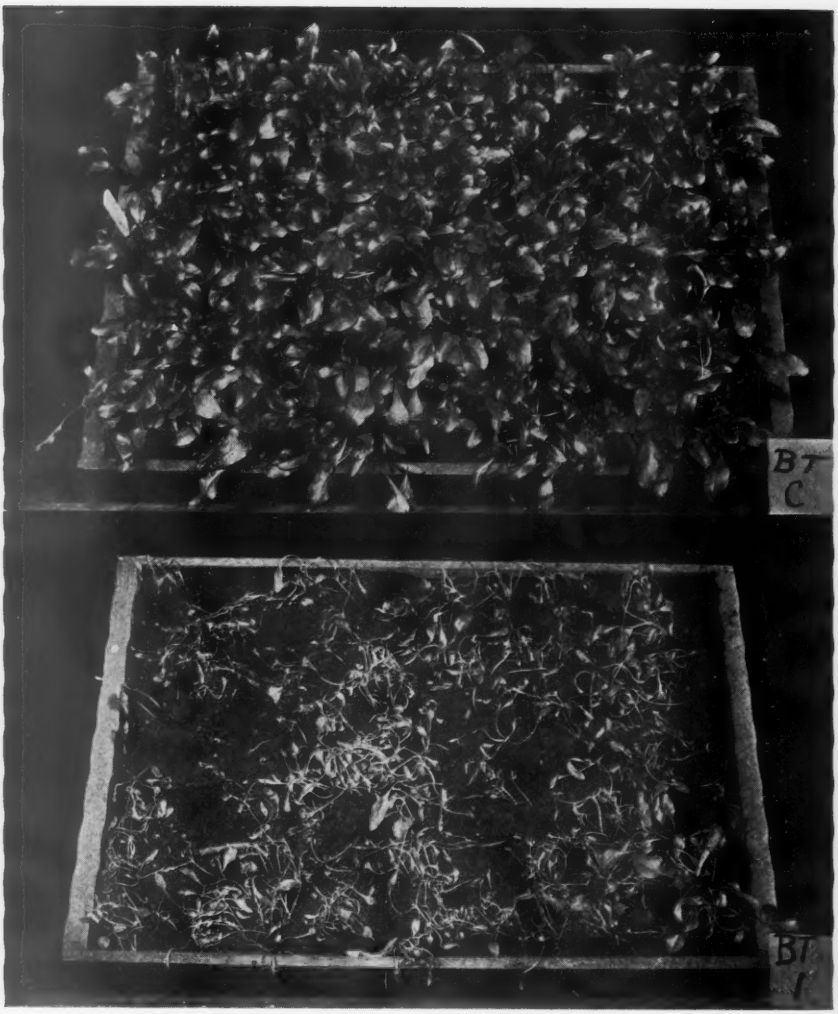

FIG. 7.—Sugar-beet plants 44 days old when treated. *Top*, untreated. *Bottom*, sprayed with aerosol that contained 2,4-dichlorophenoxyacetic acid.

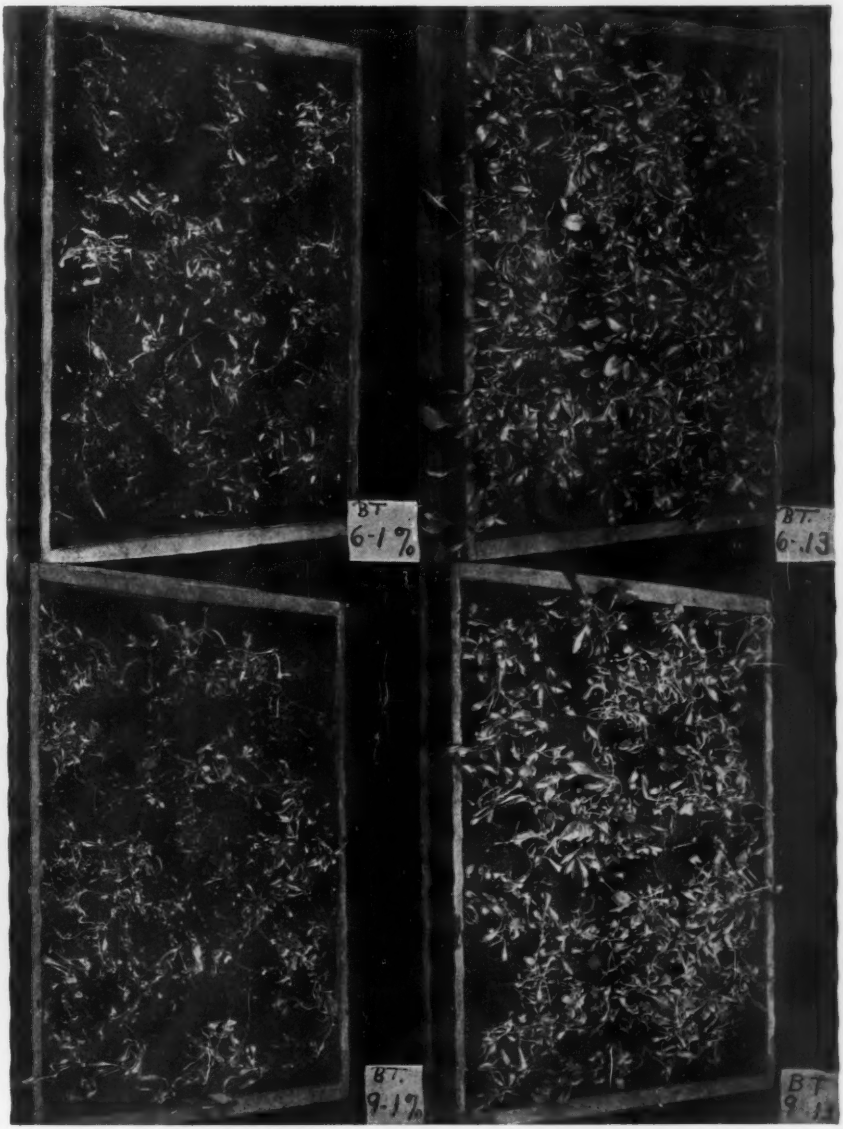

FIG. 8.—Sugar-beet plants sprayed with lanolin emulsions containing 1% (*top, left*) and 0.13% (*top, right*) of 2,4-dichlorophenoxyacetic acid and 1% (*bottom, left*) and 0.13% (*bottom, right*) of 2,4,5-trichlorophenoxyacetic acid.

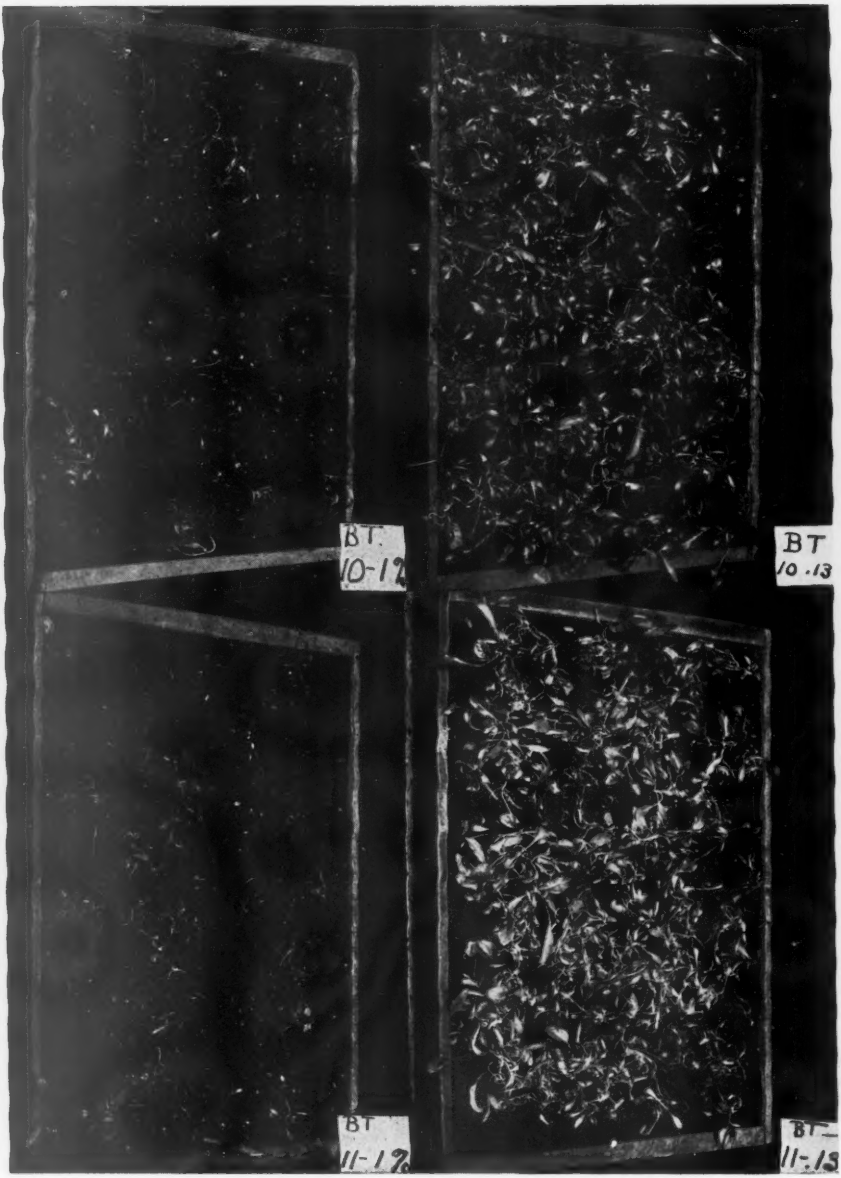

FIG. 9.—Sugar-beet plants sprayed with heavy-oil emulsions (*top*) containing 1% (*left*) and 0.13% (*right*) of 2,4-dichlorophenoxyacetic acid and light-oil emulsions (*bottom*) containing 1% (*left*) and 0.13% (*right*) of the acid.

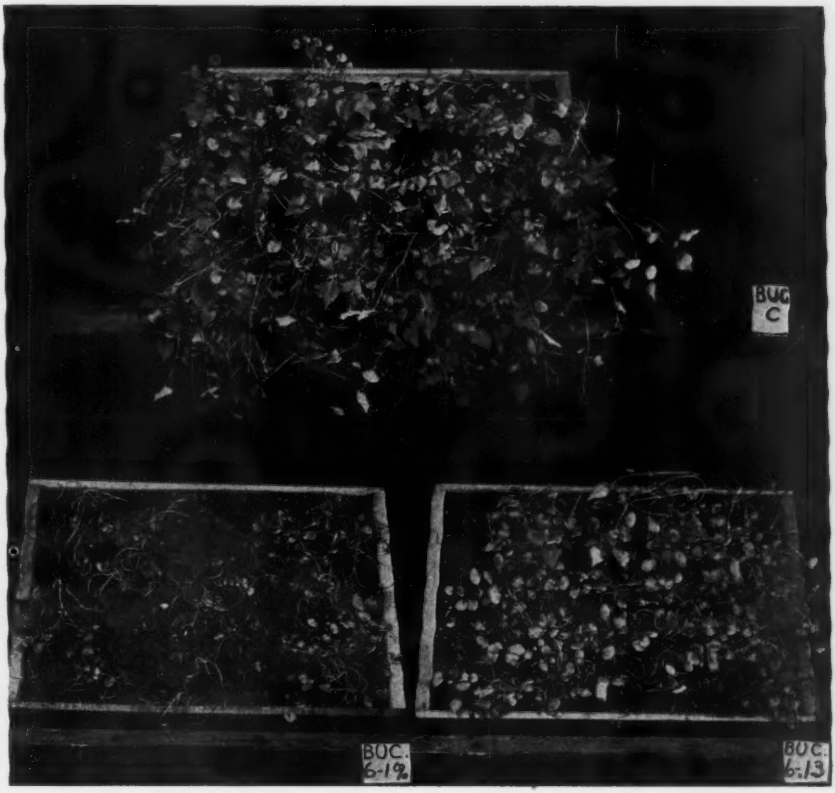

FIG. 10.—Buckwheat plants 26 days old when treated. *Top*, untreated. *Bottom*, sprayed with lanolin emulsions containing 1% (*left*) and 0.13% (*right*) of 2,4-dichlorophenoxyacetic acid.

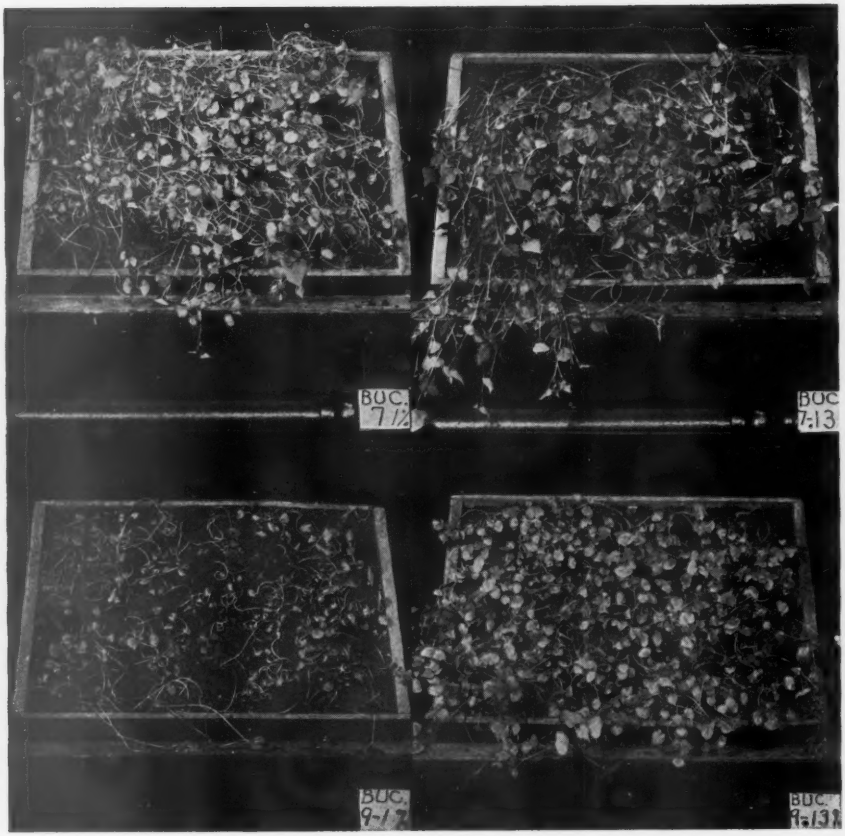

FIG. 11.—Buckwheat plants sprayed with lanolin emulsions (*top*) containing 1% (*left*) and 0.13% (*right*) of 4-chlorophenoxyacetic acid and with similar concentrations of 2,4,5-trichlorophenoxyacetic acid (*bottom*).

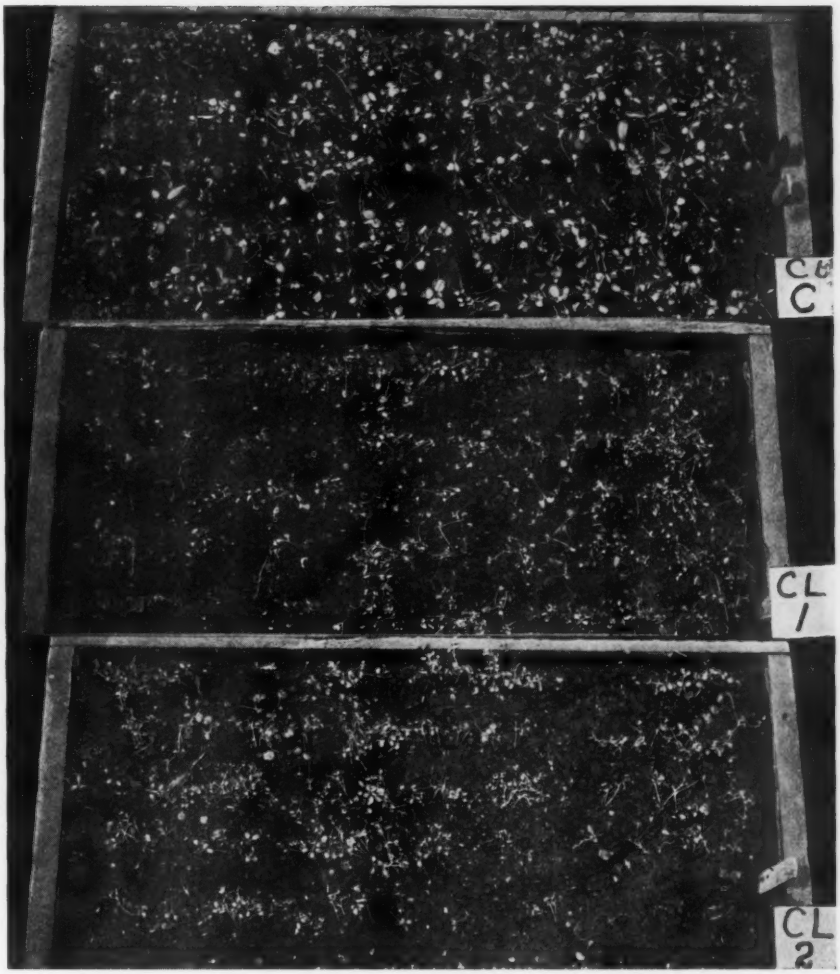

FIG. 12.—Clover plants 26 days old when treated. *Top*, untreated. *Center*, sprayed with aerosol containing 2,4-dichlorophenoxyacetic acid. *Bottom*, sprayed with aerosol containing 4-chlorophenoxyacetic acid.

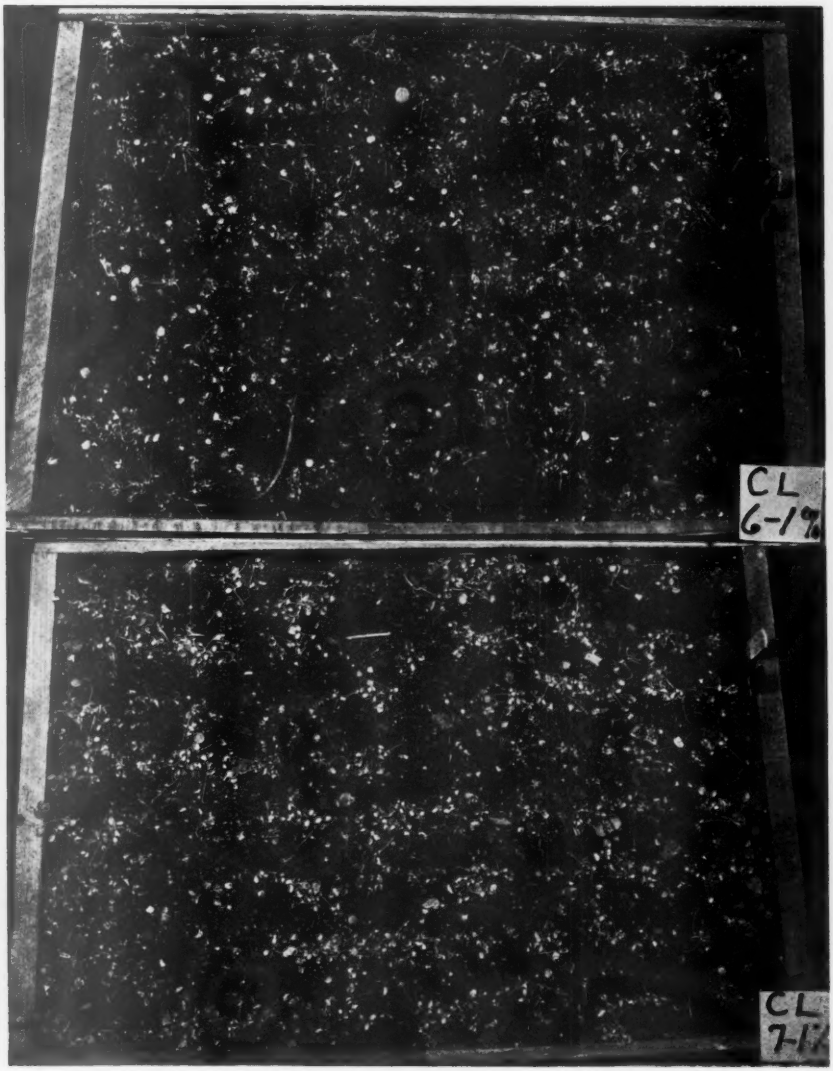

FIG. 13.—Clover plants sprayed with lanolin emulsions containing 1% of 2,4-dichlorophenoxyacetic (*top*) and 1% of 4-chlorophenoxyacetic acid (*bottom*).

FIG. 14.—Rape plants 44 days old when treated. *Top*, untreated. *Bottom*, sprayed with lanolin emulsions containing 1% (*left*) and 0.13% (*right*) of triiodobenzoic acid.

FIG. 15.—Rape plants sprayed with lanolin emulsions containing 1% (*top, left*) and 0.13% (*right*) of 2,4-dichlorophenoxyacetic acid; 1% (*bottom, left*) and 0.13% (*right*) of 4-chlorophenoxyacetic acid.

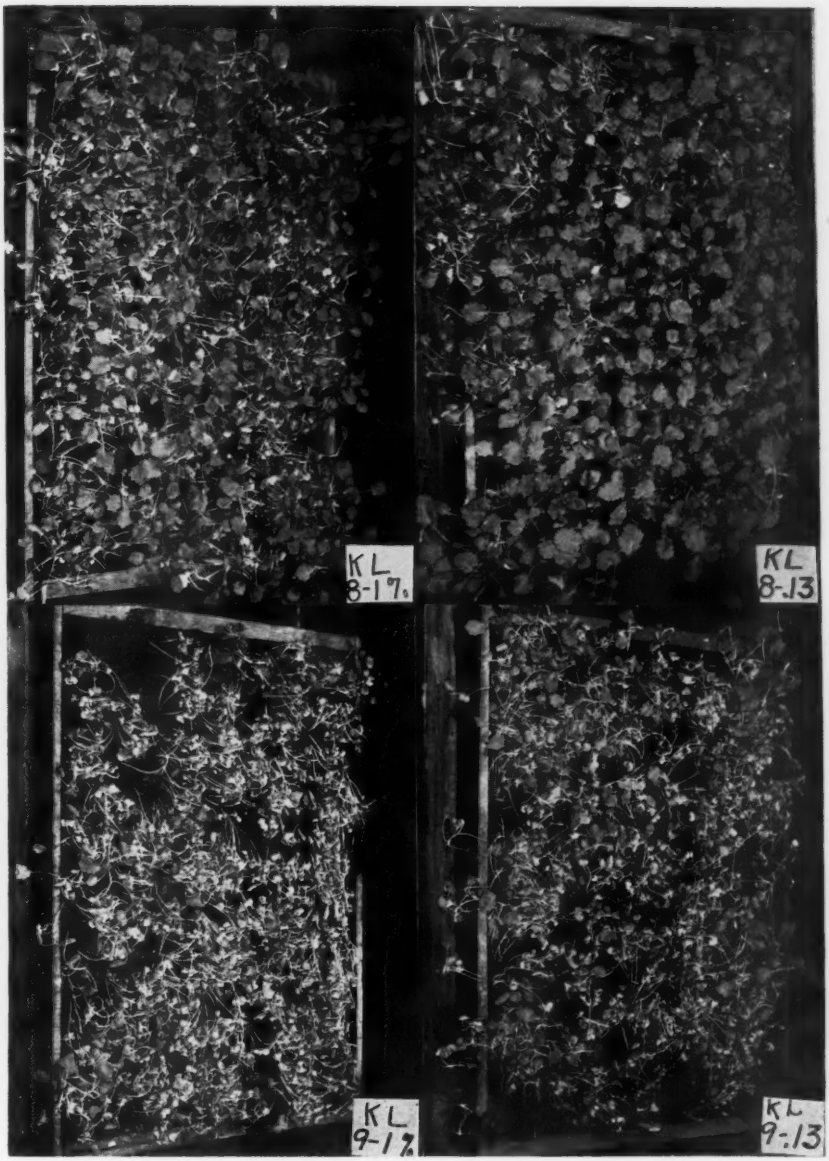

FIG. 16.—Rape plants sprayed with lanolin emulsions containing 1% (*top, left*) and 0.13% (*right*) of 2-chlorophenoxyacetic acid; 1% (*bottom, left*) and 0.13% (*right*) of 2,4,5-trichlorophenoxyacetic acid.

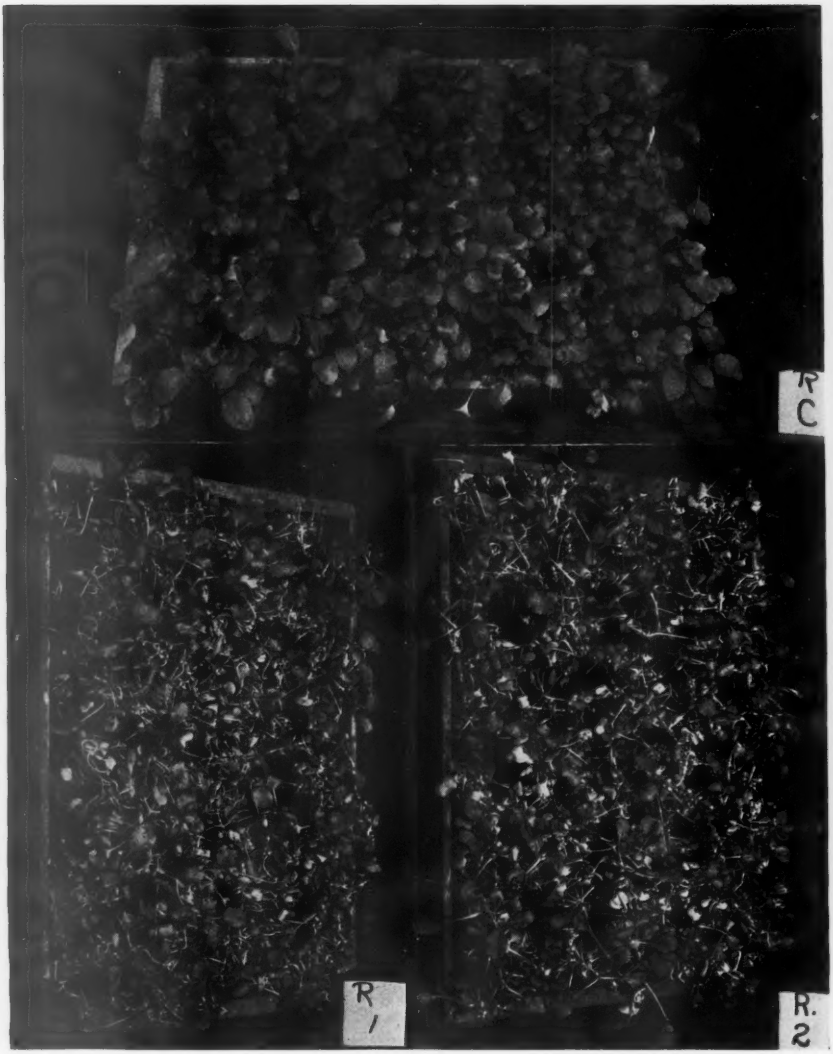

FIG. 17.—Radish plants 44 days old when treated. *Top*, untreated. *Bottom*, sprayed with an aerosol containing 2,4-dichlorophenoxyacetic acid (*left*) and 4-chlorophenoxyacetic acid (*right*).

FIG. 18.—Bean plants 26 days old when treated. *Top*, untreated. *Bottom*, sprayed with an aerosol containing 2,4-dichlorophenoxyacetic acid. Photographed 10 days after treatment. Upper plants served as "young" controls for other experiments.

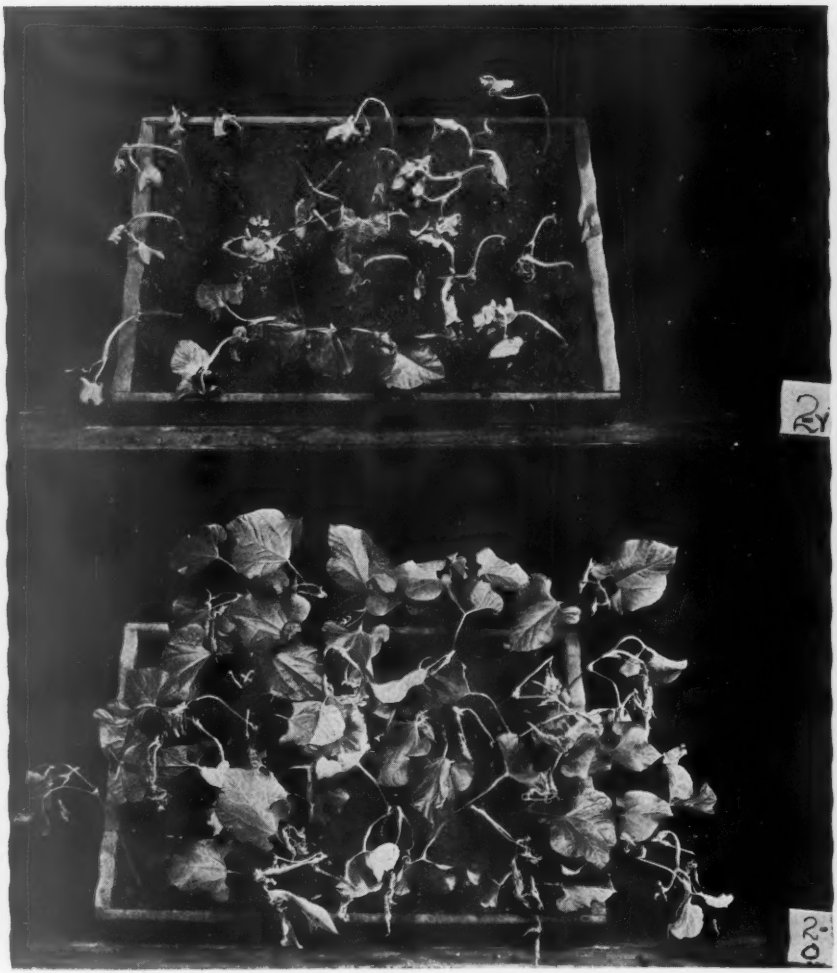

FIG. 19.—Bean plants. *Top*, sprayed 26 days after planting with an aerosol containing 6.3% of 4-chlorophenoxyacetic acid. *Bottom*, sprayed in similar way 44 days after planting. Note difference in response by plants of different ages.

FIG. 20.—Bean plants. *Top*, sprayed 26 days after planting with an aerosol containing 1.26% of pentachlorophenoxyacetic acid. *Bottom*, treated in similar way 44 days after planting. Photographs taken 10 days after treatment. Compare with fig. 19.

FIG. 21.—Bean plants sprayed 26 days after planting with lanolin emulsions containing 1% (*top*) and 0.13% (*bottom*) of triiodobenzoic acid.

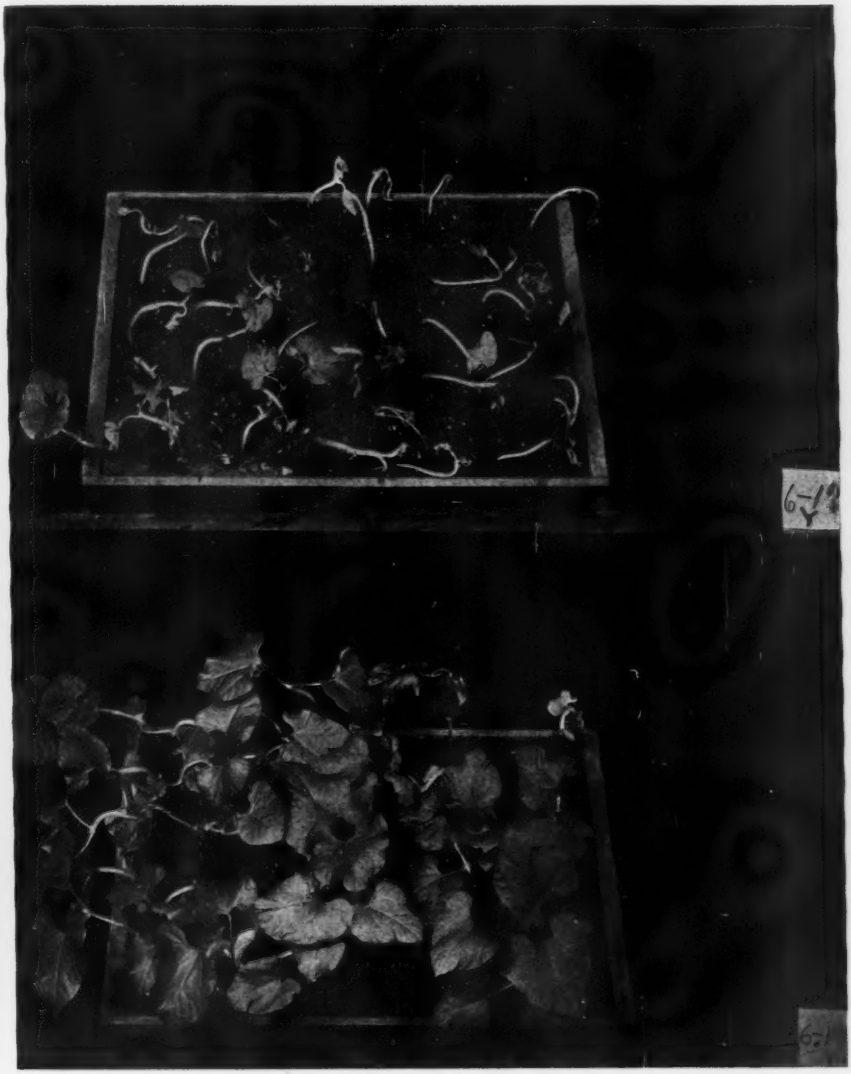

FIG. 22.—Bean plants sprayed 26 days after planting with lanolin emulsions containing 1% (*top*) and 0.13% (*bottom*) of 2,4-dichlorophenoxyacetic acid.

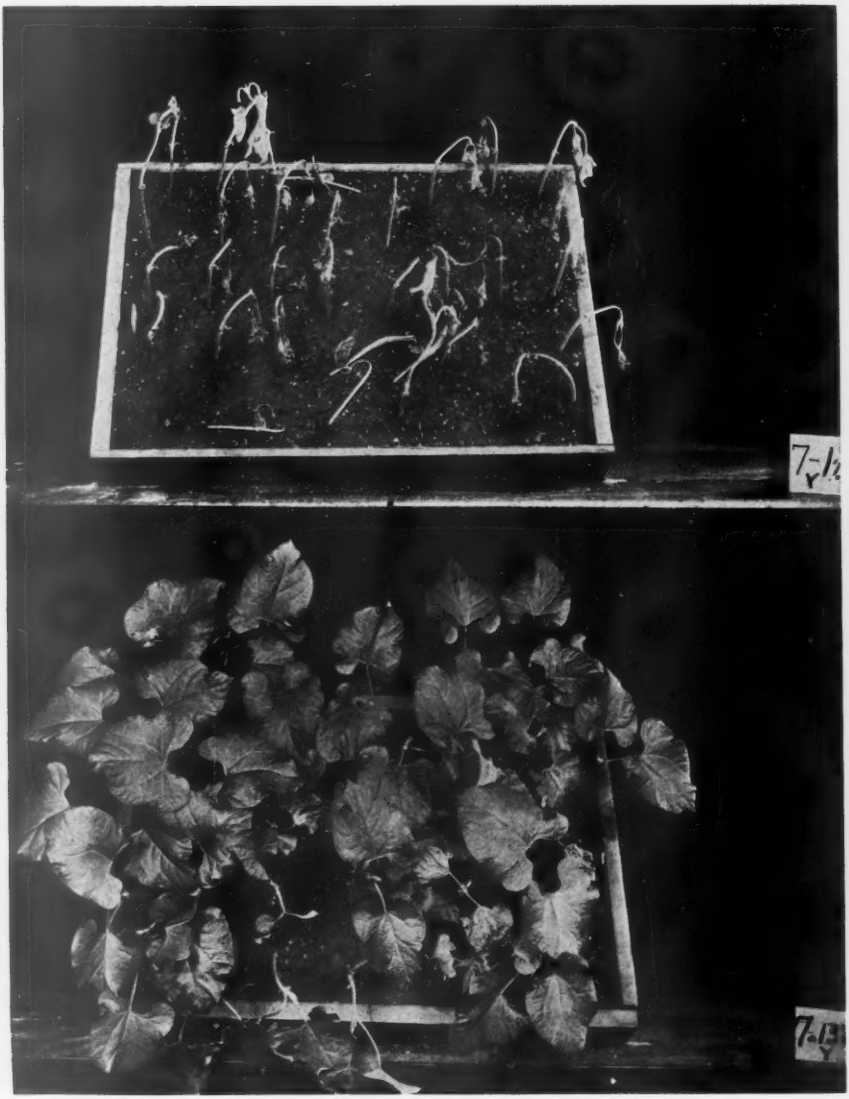

FIG. 23.—Bean plants sprayed 26 days after planting with lanolin emulsions containing 1% (*top*) and 0.13% (*bottom*) of 4-chlorophenoxyacetic acid.

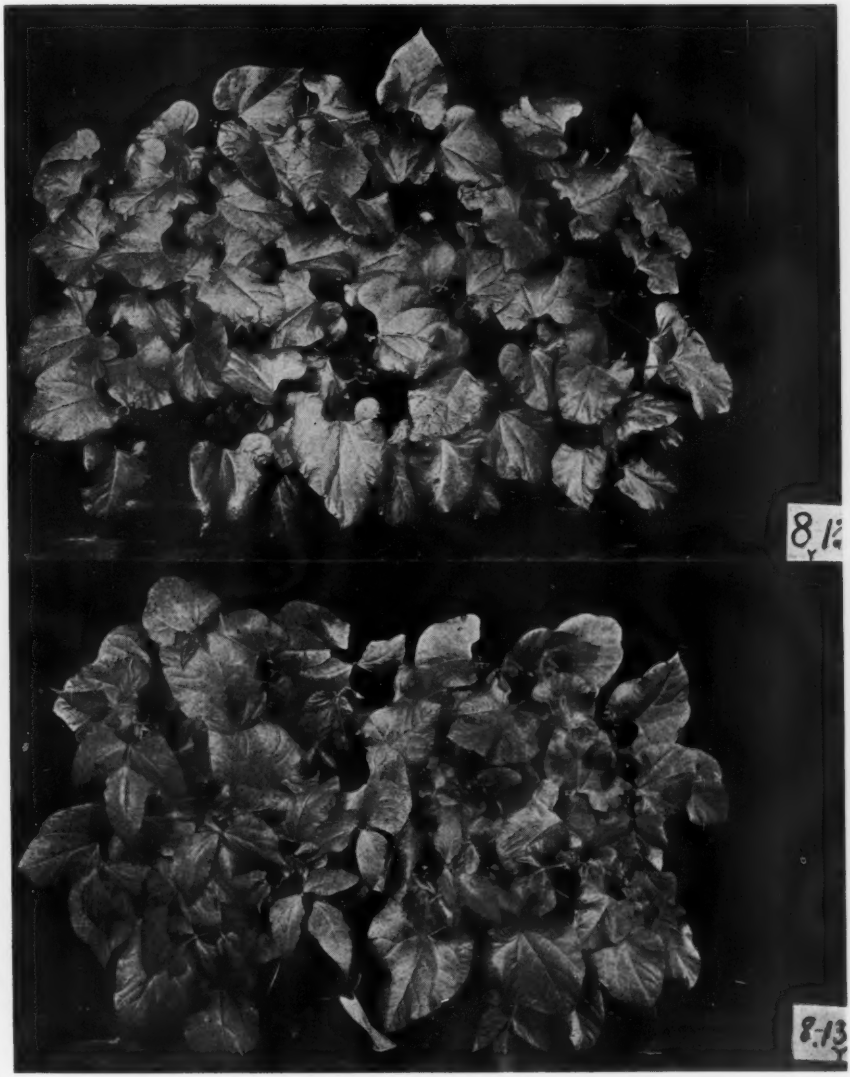

FIG. 24.—Bean plants sprayed 26 days after planting with lanolin emulsions containing 1% (*top*) and 0.13% (*bottom*) of 2-chlorophenoxyacetic acid.

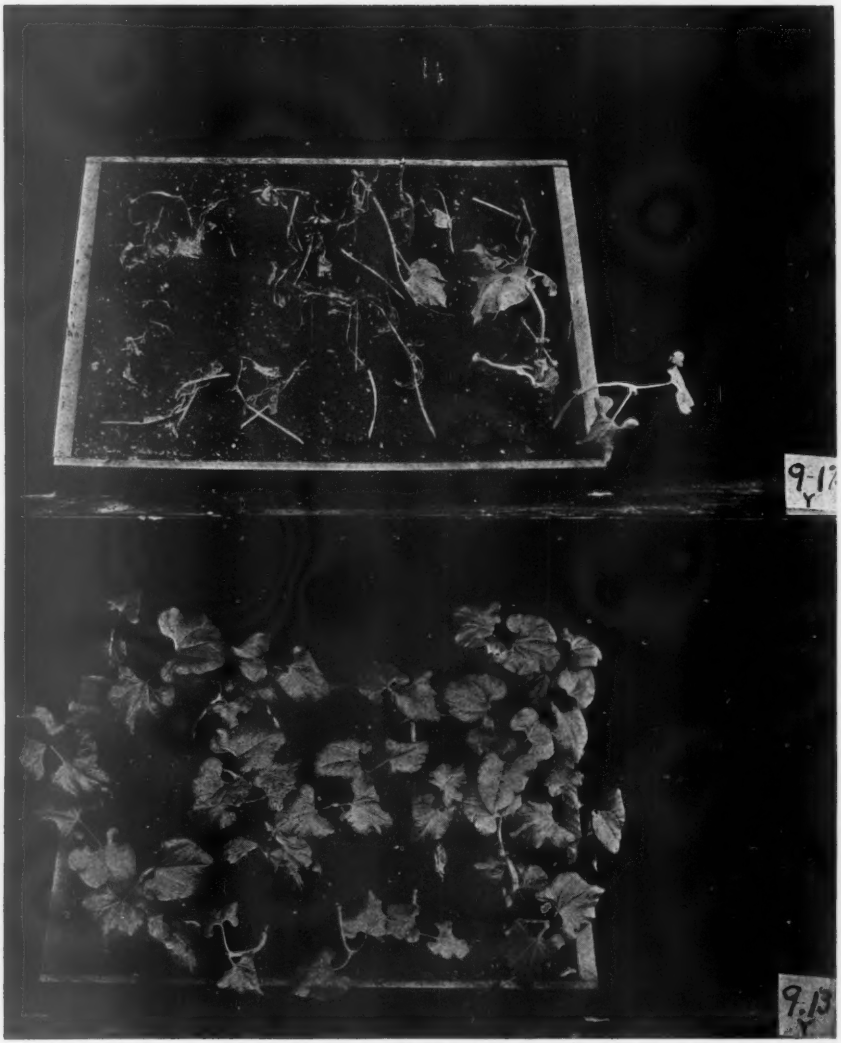

FIG. 25.—Bean plants sprayed 26 days after planting with lanolin emulsions containing 1% (*top*) and 0.13% (*bottom*) of 2,4,5-trichlorophenoxyacetic acid.

FIG. 26.—Bean plants 44 days old when treated. *Top*, sprayed with aerosol containing 2,4-dichlorophenoxyacetic acid. *Bottom*, unsprayed. Photographed 10 days after treatment. Lower plants are referred to as "old" controls.

FIG. 27.—Bean plants sprayed 44 days after planting with aerosol containing 4-chlorophenoxyacetic acid (*top*) and penta-chlorophenoxyacetic acid (*bottom*).

FIG. 28.—Bean plants sprayed 44 days after planting with lanolin emulsions containing 1% (*top*) and 0.13% (*bottom*) of 2,3,5-triiodobenzoic acid.

FIG. 29.—Bean plants sprayed 44 days after planting with lanolin emulsions containing 1% (*top*) and 0.13% (*bottom*) of 2,4-dichlorophenoxyacetic acid. Compare with fig. 22.

FIG. 30.—Bean plants sprayed 44 days after planting with lanolin emulsions containing 1% (*top*) and 0.13% (*bottom*) of 2,4,5-trichlorophenoxyacetic acid. Compare with fig. 25.

FIG. 31.—Bean plants sprayed 44 days after planting with heavy-oil emulsions containing 1% (*top*) and 0.13% (*bottom*) of 2,4-dichlorophenoxyacetic acid. Compare with fig. 29.

FIG. 32.—Bean plants sprayed 44 days after planting with light-oil emulsions containing 1% (*top*) and 0.25% (*bottom*) of 2,4-dichlorophenoxyacetic acid. Compare with fig. 31.

FIG. 33.—Bean plants dusted 44 days after planting with talc containing α-naphthyl methyl acetate (*top*) and penta-chlorophenoxyacetic acid (*bottom*). One part of growth-regulator to 500 parts talc.

FIG. 34.—Bean plants 44 days old when treated. *Top*, sprayed with lanolin emulsion containing 0.13% of 4-chlorophenoxyacetic acid. *Bottom*, dusted with talc containing 1 part of 2,4-dichlorophenoxyacetic acid to 500 parts of talc. Photographed 10 days after treatment. Some of effects which developed much later following treatment with dusts are discussed in text.

2,4-Dichlorophenoxyacetic acid, 4-chlorophenoxyacetic acid, and pentachlorophenoxyacetic acid were dispersed individually as aerosols in which the growth-regulating substances were carried in motor oil (S.A.E. 40). In producing an aerosol, 6.3 gm. of 2,4-dichlorophenoxyacetic acid were added to 50 ml. (approximately 50 gm.) of oil, and this mixture was dissolved in 150 gm. of dimethyl ether. The 4-chlorophenoxyacetic acid mixture was prepared by adding 3.15 gm. of the acid to 50 gm. of oil and dissolving this mixture in 150 gm. of dimethyl ether. To produce the third aerosol, 630 mg. of penta-chlorophenoxyacetic acid were added to 50 ml. of oil, and this mixture was dissolved in 150 gm. of ether. These mixtures were contained in small individual metal cylinders. In applying the aerosols, the cylinders were held approximately 2 feet above the uppermost leaves and directed downward so that the mist covered the entire flat of plants. Each flat was sprayed for a period of from 4 to 8 seconds.

Dusts were prepared that contained separately, α-naphthaleneacetic acid, α-naphthyl methyl acetate, penta-chlorophenoxyacetic acid, and 2,4-dichlorophenoxyacetic acid. Two concentrations of these agents were used, 1 part to 500 dust and 1 part to 5000 dust. In preparing a dust, the growth-regulating substance was first dissolved in alcohol. This solution was thoroughly mixed with the required amount of talc, and finally the paste was dried and repulverized, leaving the agent evenly distributed throughout the talc. The dust was applied to the above-ground portions of the plants by placing it in a cheesecloth bag and shaking the dust through the cloth onto the plants.

All treatments were applied on February 29, 1944. On the tenth day following treatment the photographic records were made. The plants which survived treatment were grown under greenhouse conditions for a period of from 1 to 2½ months, during which time records as to their growth and fruitfulness were made. Some of these records are presented individually near the end of this paper; others are in the general summary of results given here.

Results of trial F

Toxicity.—2,4-Dichlorophenoxyacetic acid killed beet, rape, buckwheat, radish, clover, and young and relatively mature beans within 10 days when applied at a concentration of 1% in lanolin emulsion spray. Lower concentrations (0.5, 0.25, and 0.13%) of the acid in lanolin emulsions greatly retarded the growth of these plants but did not kill all of them. Both heavy- and light-oil emulsions containing 1.0 and 0.5% of this acid killed bean and beet seedlings, while the 0.25 and 0.13% concentrations greatly retarded growth but did not kill all the plants. Oil containing the acid and dispersed as an aerosol killed seedling buckwheat, bean, rape, beet, clover, and relatively mature beans and greatly inhibited the growth of radish seedlings. 2,4-Dichlorophenoxyacetic acid was equally toxic when applied either to the more mature or to the younger beet plants. Applied in concentrations of 1% in a light-oil emulsion spray, the acid killed rice of the varieties Blue Rose and Fortuna.

2,4,5-Trichlorophenoxyacetic acid killed seedling beet, clover, and the young bean plants within 10–15 days after treatment and greatly inhibited the growth of radish, rape, buckwheat, barley, and relatively mature bean plants when applied at a concentration of 1.0 and 0.5% in lanolin emulsion. An emulsion containing 0.13% of the acid in-

hibited the growth of rape, buckwheat, and bean plants.

4-Chlorophenoxyacetic acid killed clover and seedling bean plants within 10–15 days after treatment when applied at a concentration of 1% in lanolin emulsion and clover plants when applied at a concentration of 0.13% in lanolin emulsion. In a concentration of 1% in lanolin emulsion it greatly retarded the growth of seedling rape, beet, radish, buckwheat, and the more mature bean plants and caused appreciable injury to barley. The acid effectively retarded the growth of young bean plants when applied at a concentration of 0.13% in lanolin emulsion. Dispersed as an aerosol, it killed most clover plants so treated.

2,3,5-Triiodobenzoic acid noticeably retarded the growth of rape and young and more mature bean plants when applied at a concentration of 1% in lanolin emulsion. A 0.13% concentration of the acid, applied in lanolin emulsion, resulted in slight inhibition of the expansion of the trifoliate leaves of beans. Although this compound brought about very marked growth responses, particularly of the leaves, it failed to kill the different kinds of plants to which it was applied. After a period of time many of the plants treated with this compound not only recovered and bore fruit but remained vigorous for a considerable period after untreated controls of the same age had matured and died. Some of these results are recorded below.

Penta-chlorophenoxyacetic acid was applied in oil as an aerosol. It killed the majority of seedling clover plants so treated and injured some of the leaves of young corn plants, but it failed to retard their growth an appreciable amount. Applied in talc, it did not affect the growth of bean, beet, or barley plants.

2-Chlorophenoxyacetic acid brought about some plant-growth responses (epinasty and stem bending) but failed to inhibit to an appreciable amount the growth of most plants tested.

Talc containing either 2,4-dichlorophenoxyacetic acid, penta-chlorophenoxyacetic acid, α-naphthyl methyl acetate, or α-naphthaleneacetic acid was not effective in producing toxicity symptoms in beet, bean, or barley.

The growth of corn and wheat was not noticeably affected by the application of the various substances used in these experiments. The growth of oats was retarded slightly, and of barley appreciably so, by the application of 2,4,5-trichlorophenoxyacetic acid at a concentration of 1% in lanolin emulsion. Lower concentrations of the acid applied in the same way had little apparent effect. In general, cereals proved to be relatively insensitive to the various growth-regulating substances in the manner used. Rice, however, was killed by the application of 2,4-dichlorophenoxyacetic acid emulsion to the plants and to the water over the soil in which they were growing.

GROWTH RESPONSES.—Aside from toxic effects, various types of growth responses resulted from the application of certain of the substances used. Some of these responses are of significance here, since they altered the growth of the plants in such a way as to reduce greatly the amount of fruit produced or to extend the amount of time required for the plant to come to the fruiting stage.

Application of 2,4-dichlorophenoxyacetic acid, at a concentration of from 0.13 to 1.0% in lanolin emulsion, resulted in epinastic responses and stem curvatures within a period of 2 or 3 days following treatment. Growth of the terminal buds of the various dicotyledonous plants tested was greatly inhibited, especially when the acid was applied in

concentrations of 0.25% or more. Tumors developed in the stem tissues, and, as a result, the over-all growth of the plants was greatly reduced, as was the number of fruits which developed. With the exception of slight stem curvatures, no apparent growth response or form changes resulted when 2,4-dichlorophenoxyacetic acid was applied to the foliage of the cereals except as already noted for rice and possibly barley.

2,4,5-Trichlorophenoxyacetic acid also brought about very marked form changes when applied to the dicotyledonous plants as lanolin-emulsion or oil-emulsion sprays. These were similar in outward appearance to those described in connection with the use of 2,4-dichlorophenoxyacetic acid. The application of 2,4,5-trichlorophenoxyacetic acid to the cereals failed to bring about any significant changes of form except as noted for oats and barley.

When applied to the dicotyledonous plants, 4-chlorophenoxyacetic acid inhibited the growth of terminal buds to a very marked extent. The growth responses observed were similar in appearance and effect to those reported in connection with the use of 2,4-dichlorophenoxyacetic acid. The application of low concentrations (0.13–0.25%) of the acid to seedling clover plants resulted eventually in the development of leaves which had from three to nine leaflets and in the formation of numerous roots which emerged from the hypocotyls. Later the clover plants recovered completely and grew normally.

The application of 2,3,5-triiodobenzoic acid to the dicotyledonous plants resulted in the inhibition of internodal elongation and in modification of growth of the leaves in the terminal buds. The growth of lateral buds was affected in a similar way when emulsions containing 1% of the acid were applied. Leaves of the affected buds failed to expand the usual amount. They were very small and curled, forming rosettes while the plant was in the seedling stage. They expanded slightly as the plants matured. The leaves produced later were normal. In the case of the beet, many of the leaves produced after treatment were tubular, funnel-shaped, or filiform, with the surface much pebbled. 2,3,5-Triiodobenzoic acid failed to induce the formation of stem tumors on any of the plants. The number of flowers that developed on treated plants was not appreciably fewer than the number that developed on untreated ones. However, at the end of the experiment plants treated with a concentration of 1% of the acid in lanolin emulsion were somewhat smaller in over-all size than were untreated ones. The behavior of some of the plants when appreciably older is recorded below, under treatment 5. The application of the acid did not noticeably alter the growth of the monocotyledonous plants studied.

Many of the flats of plants in this trial which were not killed by the treatments were held over and placed on greenhouse benches for further development. It is obvious that several points of great agricultural importance are shown, especially the increased longevity of some of the lots of plants and the intense green coloration developed by the foliage following some of the treatments. These observations, especially as they relate to an apparent increase in development over the controls and give indications of a decided stimulative effect, are of as great significance as are the toxicity effects.

Except as specifically indicated the following notes on bean plants were made on April 17, 1944. The exceptions relate to a few flats on which the notes were

made on May 3, 1944. The numbers used designate the same type of treatments as given in table 1.

5. *1%* (*old*).—Yellowed; one set of trifoliate leaves normal, the others curled and crumpled, or pebbled. Few flowers, few pods, one to three beans each.

5. *0.5%* (*old*).—Yellowed; many large trifoliate leaves, many small axillary shoots; those at cotyledonary node show no crumpling and are dark-green. There are a few dry pods having one to three beans each, many large green pods having one to three beans each, and a number of flowers on the axillary shoots. In general, slightly greener than controls. The plants are not so green as treatment **8.** *Dust* nor so large.

5. *0.13%* (*old*).—Yellow, many plants mature and dried, a few with axillary shoots at the cotyledonary node. Most pods dry, one to two seeds each. Some large trifoliate leaves. Slightly less mature than controls.

5. *1%* (*young*).—Primary leaves large, yellowed, somewhat recurved; epicotyls with few green leaves or leaves dead and shed. Axillary shoots at cotyledonary node. Small, very green leaves, a few young green pods, one to three seeds each. Still many flowers and young pods forming.

5. *0.5%* (*young*).—Epicotyls dead, few axillary shoots, also a few shoots from axils of cotyledons. These shoots are still green. Leaves mainly yellowed or fallen, stems dead or dying. Plants sparse in appearance.

5. *0.25%* (*young*).—Yellowed, dying, epicotyls dead; a few had produced thin shoots with small leaves. A few pods with one bean in each. Weak sprouts from axils of cotyledons.

5. *0.13%* (*young*).—Yellowed; some second internodes are elongated and have few large leaves. Axillary shoots at nodes of cotyledons and primary leaves. Leaves small, pebbled, all yellowed, except those on small axillary shoots. Few green or dry pods. Less mature than controls.

6. *0.13%* (*young*).—Still green, the primary leaves green and somewhat leathery. Epicotyls killed, with large tumors, 1 inch in length, stopping at cotyledonary nodes where large tumors have formed on some plants. Small, green, axillary shoots at cotyledonary nodes, a few in axils of primary leaves. There are flowers only at these places. A few green pods, containing one to three beans each. Plants still actively vigorous; new green leaves show little deformation.

6. *0.13%* (*old*) (*May 3, 1944*).—Many axillary shoots, some of them with tumors, mostly without. Primary leaves not very large but still green. Short axillary shoots green, with green pods having one to three beans each. Larger trifoliate leaves mottled yellow. Plants rather open, not very bushy and still appear decidedly green. Less bushy than **7.** *0.13%* (*old*). More pods than controls, some of them still green, some dried.

7. *0.13%* (*young*).—No large trifoliate leaves. Those which are present still very green. Most of the terminal shoots were killed; others have large galls which are corky, many extending to the cotyledonary nodes, none below. The lateral shoots are dwarfed with some bloom, but most of the pods are aborted. Many leaves filiform; no compound leaves of full size. Most of the primary leaves still dark-green, leathery, but not of unusual size. Plants as a whole appear very green.

7. *0.25%* (*old*).—Still very green, except a few primary leaves and the old, full-sized, trifoliate leaves which are yellowed. Apical buds of main stems and also buds present in the axils of trifoliate leaves at time of spraying became tumors. Many new shoots in axils of primary leaves; most of them with deformed, sometimes filiform, leaves. These shoots are blooming and sometimes have green pods, many of which are abortive. Axillary shoots at cotyledonary node with very green leaves which are slightly deformed. Many plants in bloom and with large green pods having one to three beans each, although some are large and empty. Plants have a decidedly bushy appearance. They are vigorous except for many deformed leaves. General appearance very green with many buds and blossoms.

7. *0.13%* (*old*) (*May 3, 1944*).—Plants green and taller than **7.** *0.13%* (*young*); many large trifoliate leaves which are slightly yellowed. Tops and axillary shoots which were present at nodes of large trifoliate leaves when sprayed are now oval, fleshy, corky tumors with apical rosettes of scales. A few plants have much-branched shoots instead of tumors. These shoots have intense green leaves which are rather small and sometimes filiform. A few axillary shoots at cotyledonary nodes are mostly small with leaves less deformed. Many large green pods, one to five beans each. Some flowers and a few buds.

8. *1%* (*young*).—Greener than plants treated at 0.5% strength. Axillary shoots with many

small deformed leaves. A few abortive flowers and pods. Some buds with one to two seeds each. Many leaves filiform. General appearance very twiggy.

8. *0.5%* (*young*).—Yellow. Epicotyls thin, dying, with many of the leaves filiform or with broad midveins and no blade. Weak shoots in axils of cotyledons.

8. *0.25%* (*young*).—Yellow, not so mature as controls. Epicotyls slender; many leaflets of trifoliate leaves narrowly triangular, deformed, and pebbled. Pods dry, one to two beans each. Stems green.

8. *0.13%* (*young*).—Yellow, but less so than controls. Epicotyls slender; leaves small, slightly deformed. Very little branching from axils of cotyledons or leaves. Few pods, usually with one seed.

8. *1%* (*old*).—Greener than controls, but plants are not so green as treatment **8.** *Dust*. Many large trifoliate leaves, some of which are yellowed, others very green. Many small green axillary shoots.

8. *0.5%* (*old*).—Not much deformation of plants, but they are much greener than controls. Stems green. A few dry pods.

8. *0.25%* (*old*).—Similar to *0.5%* (*old*) but still greener.

8. *0.13%* (*old*) (*May 3, 1944*).—Plants open, sparse, about like controls, but slightly greener. Many green pods, a few of them mature except at nodes of primary leaves and cotyledons.

11. *0.13%* (*old*).—Still much greener than controls. Second internode and internodes above elongated in many cases. A few have tumors on them. Leaves not much deformed, some trifoliate leaves large and green. Very few pods; most of them are green, with one to three seeds each. Stems green. Some tumors on petioles of older leaves.

1. *Dust*.—Slightly greener than controls; apparently more pods.

2. *Dust*.—Similar to treatment **1.** *Dust* but more small, dark-green, axillary shoots.

3. *Dust*.—Greener than controls, more pods which are still fairly green, three to five beans each. Stems green. Compared with controls, less mature, slightly more vigorous.

7. *Dust*.—Plants greener than controls, many still growing actively. There are a few dry pods and some green ones having one to four seeds each. More growth than controls and still very much greener.

8. *Dust*.—Plants larger than controls, greener, and with many small axillary shoots. More pods, some of them still green. A few flowers still present.

Control (*young*).—Plants somewhat green, but primary leaves are yellow, as are also many of older trifoliate leaves. Stems still green. Few dry pods; few axillary shoots, those present being at nodes of cotyledons or primary leaves.

Control (*old*).—Plants yellow; most of primary leaves curled or shed, the large trifoliate leaves yellowish. Many mature pods, one to four seeds each. Stems collapsing and dying, mostly fallen over.

Summary

1. On the basis of the records of many experiments which demonstrated the relative toxicity of some of the growth-regulating substances and the differential sensitivity shown by plants in relation to them, the suggestion was made that greater attention be directed toward more critical study of these effects and their possible significance to agricultural and industrial practice. Following out this idea, it was specifically suggested that attention be given to the possible significance of these compounds in a program for national defense.

2. Experiments, in addition to work in progress, looking to both ends were carried out for a number of years. Summary results of certain of the specific experiments carried out from August, 1941, to May, 1944, are given. Others may be presented at some future time.

3. When attention was called to some of the chlorophenoxyacetic and iodobenzoic acids, additional critical experimental work was at once centered around some of them.

4. The 2,4-dichlorophenoxyacetic, 2,4,5-trichlorophenoxyacetic, and 4-chlorophenoxyacetic acids proved extremely toxic when applied under greenhouse conditions to the aerial parts of sugar-beet, radish, rape, kidney bean, red clover, and buckwheat plants. Emulsions of these compounds, when applied to the foliage of rice plants and to the water

over the surface of the soil in which they were growing, were highly toxic.

5. Various other substances were effective as plant toxicants or in modifying growth when sprayed on the plants as lanolin or oil emulsions or when used as aerosols.

6. The concentrations of these agents required to kill or markedly to reduce the rate of growth of the plants ranged between 0.13% and 1.0% when the acids were applied as emulsion sprays. Penta- and 2-chlorophenoxyacetic acids were less toxic than 2,4-dichlorophenoxyacetic, 2,4,5-trichlorophenoxyacetic, and 4-chlorophenoxyacetic acids.

7. Dusts containing growth-regulating substances were relatively ineffective as applied in these tests. It was suggested that, on the basis of these results, the addition of a hygroscopic agent to the mixture of talc plus a growth-regulating substance might allow for the absorption of moisture and thereby place a sufficient amount of the agent in a form to induce growth responses and even severe injury.

8. In general, cereals were relatively insensitive to growth-regulating compounds as used in these experiments. Rice was killed by the application of 2,4-dichlorophenoxyacetic acid to the water surrounding the plants as well as to their leaf surface. Barley also proved to be somewhat sensitive to some of the mixtures but less so than most of the dicotyledonous plants tested.

9. Aside from the toxic effects, it was established that the application of 2,4-dichlorophenoxyacetic acid, and some of the other substituted chlorophenoxyacetic acids, brought about responses by the dicotyledonous plants that were outwardly different in appearance from those that resulted from the application of 2,3,5-triiodobenzoic acid. On the basis of the results obtained, it appeared that 2,4-dichlorophenoxyacetic and 2,4,5-trichlorophenoxyacetic acids would be outstandingly useful as herbicides or as differential herbicides.

10. In connection with the experimental work then under way, in March, 1944, plots were laid out at Beltsville, Maryland, to study the use of growth-regulating substances as herbicides when applied to field-grown plants.

11. The results of the various experiments carried out, together with many photographs, some of which accompany this paper, were conveyed to those authorized to receive such reports (13).

12. The use of growth-regulating substances in the control of weeds and other vegetation has now become widely publicized and exploited. Some of the other results of these experiments also are finding their application in agricultural and industrial practice.

DEPARTMENT OF BOTANY
UNIVERSITY OF CHICAGO
AND
BUREAU OF PLANT INDUSTRY, SOILS
AND AGRICULTURAL ENGINEERING
BELTSVILLE, MARYLAND

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THE COMPARATIVE MORPHOLOGY OF THE FAGACEAE
I. THE GENUS NOTHOFAGUS[1]

LADEMA MARY LANGDON

Introduction

Although the anatomy and floristic features of the Betulaceae have been studied more or less intensively by a number of investigators, including recent detailed treatment of their inflorescence anatomy and morphology by ABBE (1, 2), similar studies involving the beech-oak-chestnut branch of the Fagales have been, since the early taxonomic and phylogenetic treatises (16, 18, 29, 30, 34), conspicuously meager. RENDLE (33), SCHWARTZ (35), and CAMUS (12) gave more complete comparative accounts of generic differences in this family than are found in other recent studies of the group, but in all three—as in earlier accounts—there is notable an inadequate, almost superficial, treatment of other than external taxonomic characters and a genuine scarcity of reliable material illustrative of the internal morphology of either the vegetative or the reproductive structures.

BENSON's conclusions, based upon such features of the megagametophyte as could at the time (4) be ascertained in the Betulaceae and Fagaceae, confirmed

[1] A part of this report was presented before the General Section of the Botanical Society of America at Boston, December, 1946. The investigation has been aided by grants from the Penrose Fund of the American Philosophical Society.

EICHLER's (16) and PRANTL's (29, 30) deductions based on their floral morphology, i.e., that the Cupuliferae were to be regarded as less specialized than the Betulaceae. This view has received support from anatomical studies (3, 37) as well as from more recent investigations in the floral morphology of certain of the Castaneae (6). In general, the more recent researches in inflorescence anatomy and morphology in families of the Amentiferae (8, 19, 22, 27) have emphasized specialization in these forms, and reduction, either from the Ranalian stock along the line of the Celastrales and the Sapindales or from the Rosales through the Hamamelidales.

RENDLE's taxonomic studies (33), on the other hand, support the theory—as the one most in accord with present knowledge—that the Fagales, along with the Casuarinales and Juglandales, are surviving representatives of diverging lines of development from some early angiospermous type or types now extinct, very likely with bisexual hypogynous flowers and bractlike perianths. On the basis of his studies, ABBE (1, 2) has proposed, as a hypothetical type ancestral to the Betulaceae, a form characterized by a series of racemously arranged cymose inflorescences, each composed of a median and two lateral triflorous groups, the individual flowers hermaphroditic and epigynous. The Fagaceae are mentioned as exhibiting a combination of characters suggestive of this type.

The comparative survey of the sporophytic characters of species of the existing genera of the Fagaceae, of which the present studies of *Nothofagus* are a section, has, as its major objectives, a clearer definition of the generic characters of the entire beech-oak-chestnut assemblage as well as the acquisition of additional data bearing on the ancestry of the amentiferous angiosperms. For some time it has been recognized that there is no group of flowering plants in which greater confusion exists in relation to the limits of genera and species than the Fagaceae; and it has become increasingly evident that thoroughgoing morphological investigations, involving internal as well as external characters, would be of material assistance in indicating clear lines of distinction by which one may legitimately break up large complexes in this family into a reasonable number of distinct genera.

DISTRIBUTION OF NOTHOFAGUS

The genus *Nothofagus*, formerly considered to be a section of the genus *Fagus* —designated *Nothofagus* (false beech) by BLUME (9), BENTHAM and HOOKER (5), SOLEREDER (38), and KRASSER (23) and proposed as a genus distinct from the northern section of the beeches by the Swedish botanist OERSTEDT (28)—has been for many years recognized as comprising those beeches dominating the forests of ridges and valleys from 4000 to 700 meters altitude in extratropical South America, Australia, and New Zealand. Exclusive of the species recently reported from the Netherlands New Guinea, sixteen species have been recognized as valid in this genus; eight occur in antarctic South America, five in New Zealand, and three in southeastern Australia and Tasmania.[2] Under favorable

[2] Two theories have been current relative to the origin of the northern and southern branches of the Fageae, both receiving support from the data of paleobotany: (*a*) the hypothesis supported by BERRY (7) of a common ancestry for the northern and southern beeches and the geographical divergence of these two branches from a single area—very probably western Asia—culminating in the structural differentiation and geographical segregation characterizing the living representatives of *Nothofagus;* (*b*) the alternative hypothesis of an independent emergence of *Nothofagus* on the Antarctic continent and its northward migration by

conditions of moisture, temperature, and exposure to wind, *Nothofagus* becomes a large tree 90–200 feet in height, with a trunk 2–5 feet in diameter, frequently flanged or spur-buttressed at the base, the latter character being especially well developed on those evergreen species growing in forests subject to very heavy rainfall (**14, 15, 31**). As in other members of the beech-oak-chestnut family, however, species of *Nothofagus* growing at the higher altitudes gradually become dwarfed as they ascend until, above the 400-meter elevation, they form stunted forests of shrublike trees.

An interesting altitudinal zonation marks the distribution of the beeches of New Zealand (**14**). *Nothofagus solandri* (Hook. f.) Oerst. and *N. truncata* (Col.) Cockayne occur chiefly as lowland-montane forms, *N. fusca* (Hook. f.) Oerst. generally occupies an intermediate range up to 1000–2000 meters elevation, and *N. cliffortioides* (Hook. f.) Oerst. and *N. menziesii* (Hook. f.) Oerst. frequently ascend into the upper subalpine belt, even to the timber line.

In antarctic South America a latitudinal as well as an altitudinal zonation is associated with the distribution of the beeches, which have been reported (**15, 17, 31**) from a point on the west coast of Chile about latitude 33°, southward through western Patagonia and Tierra del Fuego. The most northern species is *N. obliqua* (Mirb.) Blume, which, according to ELWES and HENRY (**17**), was one of the principal trees in the primeval forests formerly clothing the lower slopes of the Andes at about latitude 35°. In the forest country south of the Maule River, *N. obliqua* occurs in association with *N. dombeyi* (Mirb.) Blume, these two species providing the principal timber supply of Chile. In the forests of Chillan (lat. 37°), *N. obliqua* grows up to 4000–5000 feet and is replaced at the higher elevations by *N. antarctica* (Forst.) Oerst. and *N. pumilio* (Poep. et Endl.) Krasser, the former being one of the widely distributed species, especially at high elevations, on both the Chilean and the Argentine sides of the Cordilleras from latitude 38° to Tierra del Fuego. *Nothofagus procera* (Poep. et Endl.) Oerst., a species illustrated and described in some detail in this paper, is one of the less common of the South American beeches. It is scattered in the forests of Chile (lat. 35°–36°), where it exhibits about the same altitudinal range as *N. obliqua*.

Recently, important additions to the beeches of the southern hemisphere—extending their present range of distribution from the antarctic to the highlands of the tropical zone[3]—have been reported from the collections of the Archbold New Guinea expeditions of 1937–1938, constituting part of the Netherlands New Guinea Herbarium of the Arnold Arboretum of Harvard University. These beeches, the first *Nothofagus* species to be reported from the East Indian area, are described by collectors BRASS and VERSTEEGH as large trees 30–40 meters in height, with trunks over 1 meter in diameter, dominating the forests of ridges and valleys from ±3100 meters altitude down to 2300 meters in the "Bele" River section northeast of Lake Habbema (lat. approximately 4.09° S.).

Certain of these interesting collections

three separate routes into South America, Tasmania, Australia, and New Zealand.

A recent proposal by the Russian geographer WULFF (**41**) is that a primary type, ancestral not only to the beech line but also to the chestnuts and possibly the oaks, may be represented in the modern flora by the genus *Pasania*, which includes more than a hundred species distributed in the islands of the Malay Archipelago.

[3] CAIN (**11**) has classified the Fageae (*Fagus* and *Nothofagus*) as among forms illustrating a type of bipolar distribution with species occupying essentially boreal and austral centers but completely absent from intervening tropical stations.

have been made available for examination and description in connection with this study,[4] and three different species are illustrated either in habit (figs. 14–17) or in details of anatomical structure (figs. 19, 28–33, 34–37).

Investigation

Material and methods

Although the distribution and general ecology of the known species of southern beeches, as also their taxonomic problems, have been dealt with by several investigators (**5, 9, 10, 14, 15, 17, 20, 21, 28, 31, 32, 39, 41**), very little, other than of their external taxonomic characters, is known about the morphology of these important members of the beech series—even less, in fact, than in other sections of the Fagaceae.

Most of the specimens furnishing the basic materials for the present studies were secured as loans from the extensive herbaria of the Arnold Arboretum and from the Gray Herbarium of Harvard University. Herbarium specimens have been supplemented by shipments from the Golden Gate Park, San Francisco, California, and by two sets of fixations from the Royal Botanic Gardens, Kew, England, and from the Royal Botanic Garden of Edinburgh, Scotland.

For the histological and anatomical features of inflorescences, flowers, and fruits substantially the same technical procedure has been followed in imbedding, serial sectioning, and staining as was reported in an earlier paper (**24**). In the preparation of the detailed pen drawings illustrative of the foliage, flowering, and fruiting habit of representative evergreen and deciduous species (figs. 1–17), the author has had the professional assistance of Miss Stella Dernoga of the Art Institute, Baltimore.

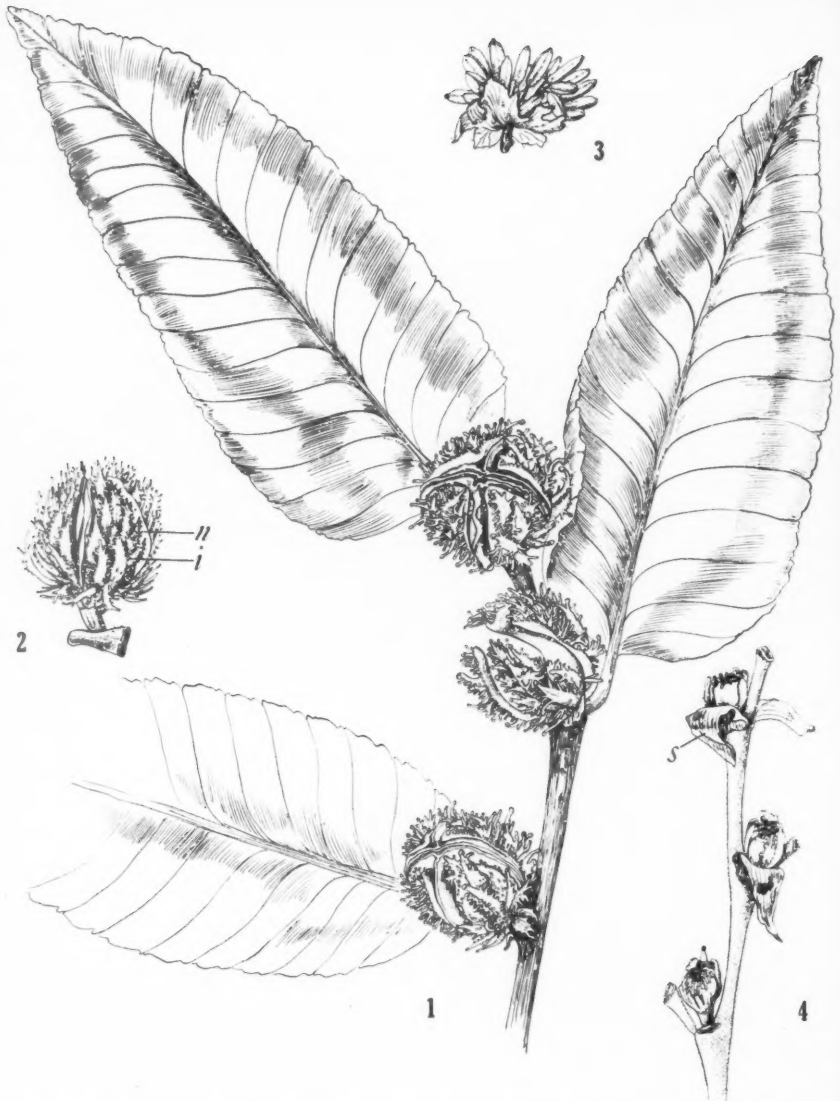
[4] Duplicate material of the New Guinea beeches was sent to Dr. A. Pulle, University of Utrecht, for study, from the Arnold Arboretum, prior to the outbreak of the war. Although to date no report has been received from Pulle, recent reports indicate that one of the New Guinean species of *Nothofagus* was published by Japanese botanists in Java during the war years and incorrectly classified as a new genus of the Hamamelidaceae.

Morphological characters

Various natural systems of classification of the Fagaceae or Cupuliferae have been proposed. In most of them the beeches of both northern and southern hemispheres are segregated as a natural group or subfamily, the inflorescences of which are described as simple one- to many-flowered dichasia in the axils of the leaves; the male flowers either in dense slender-stalked clusters or in short one- to three-flowered cymes; the female (one, two, or three in number) incompletely inclosed by a two- to four-lobed bracteolate involucre, e.g., the Fagineae of Oerstedt (**28**) and the Fageae of Prantl (**30**). Two genera are recognized—*Fagus* and *Nothofagus*.

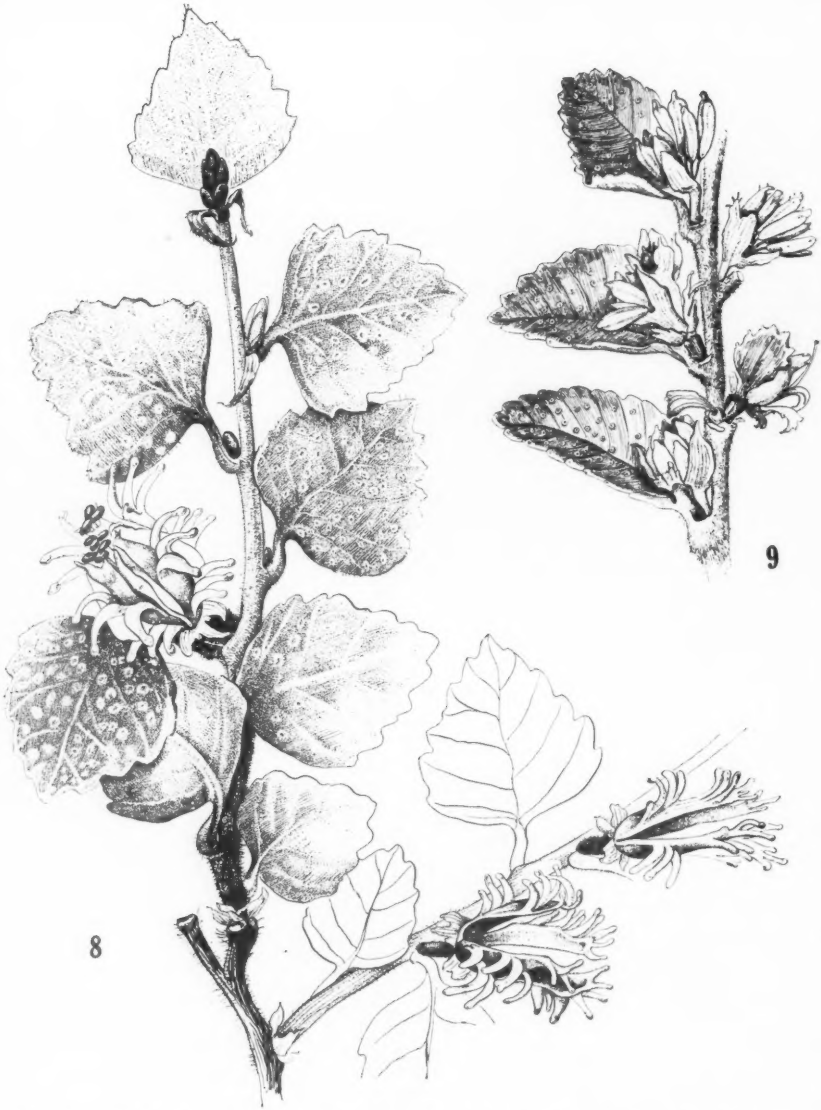
Nothofagus comprises those monoecious, occasionally dioecious, shrublike or arborescent species, with simple two-ranked, generally very small leaves; some species are deciduous, others evergreen in habit.

Leaf characters.—In the six deciduous species, all South American with the exception of the Tasmanian species *N. gunnii*, the leaves (1.5–6.5 cm. long and 1–3 cm. wide) are soft in texture, generally folded in the bud along the lateral nerves (fig. 5), and either crenate or serrate in margin, with variable degrees and types of serration in the different species. The large multicellular dermal glands, so conspicuously identified with the leaves of the evergreen beeches, may or may not occur in association with the leaves of the deciduous species. In *N. antarctica* (fig. 5), as in *N. procera* and *N. pumilio*, resinous papillae are distributed on both

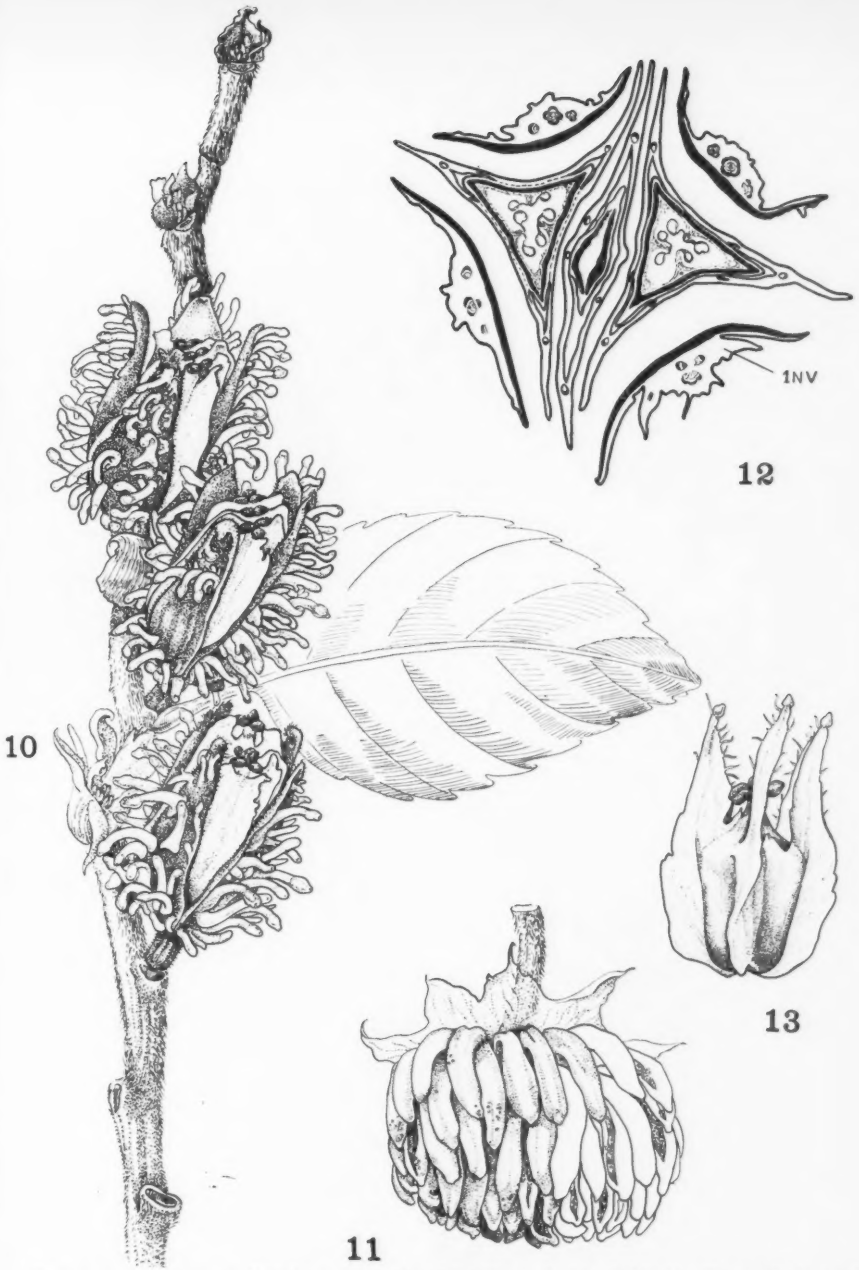


FIGS. 1–4.—*Nothofagus procera*, Chile. Fig. 1, fruiting habit; nutlets, three in number, closely invested by four-valved cupule; valves with leafy pinnatifid appendages bearing stalked glands. ×1.7. Fig. 2, young fruiting head; *n*, wing of nutlet; *i*, segment of lobular cupule. ×1.5. Fig. 3, single staminate floret. ×2. Staminate flowers in this species may be borne singly or in groups of three. Fig. 4, three pistillate inflorescences, upper two with associated stipules (*s*). ×1.5.

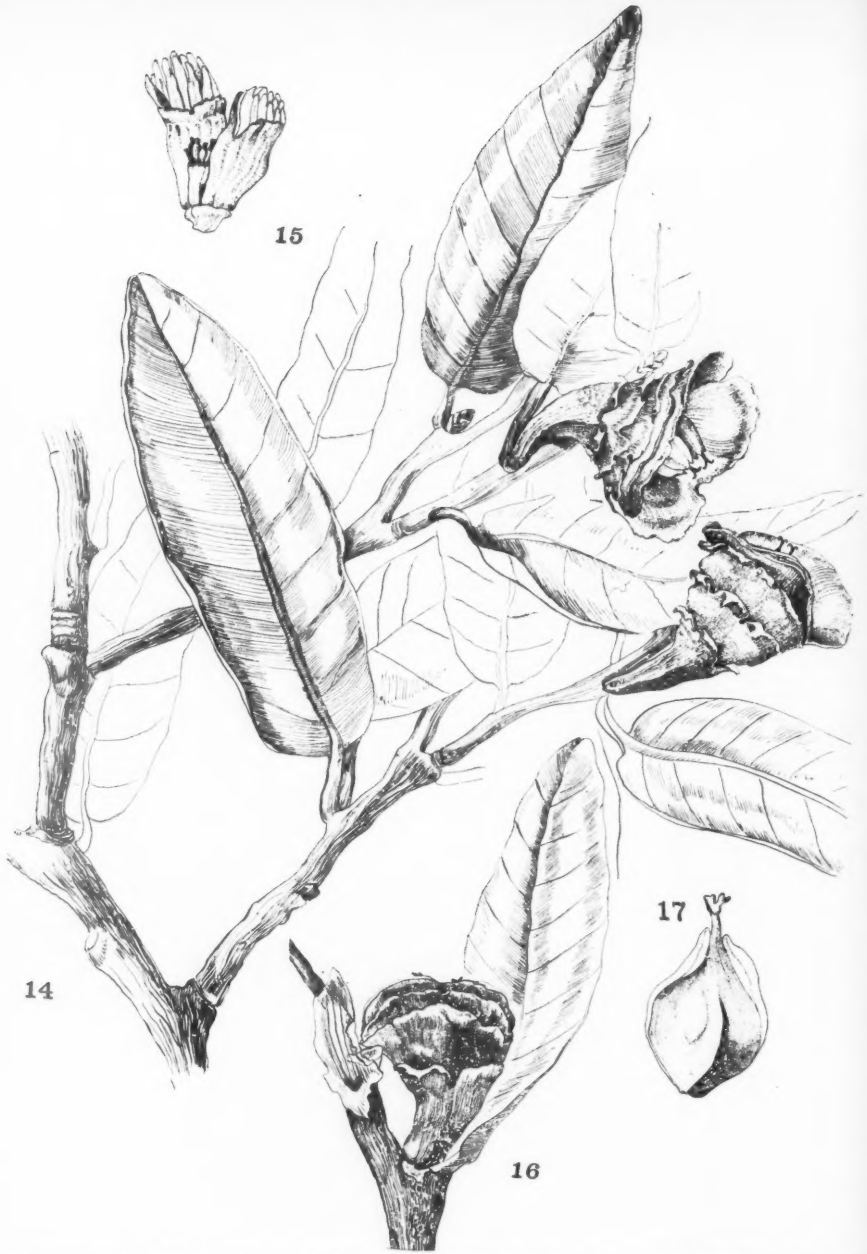
FIGS. 5–7.—*Nothofagus antarctica*, Chile and Argentina. Fig. 5, foliage and flowering habit (staminate flowering group). ×2.6. Fig. 6, pistillate inflorescences, upper group with associated stipules. ×4.6. Fig. 7, cupule-inclosed fruits. Cupule inclosing nutlets is four-valved, each valve with three to five very short transverse scarious scales. ×2.6.



FIGS. 8–9.—*Nothofagus cunninghami*, Tasmania. Fig. 8, foliage and fruiting characters, in particular conspicuously glandular character of both foliage leaves and involucral scales. ×2.6. Fig. 9, flowering shoot bearing both staminate and mixed inflorescences, also solitary pistillate floret. ×2.6.



FIGS. 10–13.—Figs. 10, 11, *Nothofagus moorei*, Australia. Fig. 10, fruiting habit. ×3. Fig. 11, single staminate floret, showing characteristic form and mode of dehiscence of anthers in *Nothofagus*. ×3. Figs. 12, 13, *Nothofagus menziesii*, New Zealand. Fig. 12, transection of nutlets with inclosing cupule valves; shaded areas indicate distribution of sclerotic tissue within pericarp and cupular valves. Fig. 13, single nutlet, illustrating characteristic winged character of fruits in *Nothofagus;* wings of this species project well above stigma lobes and are gland-tipped. ×4.6.

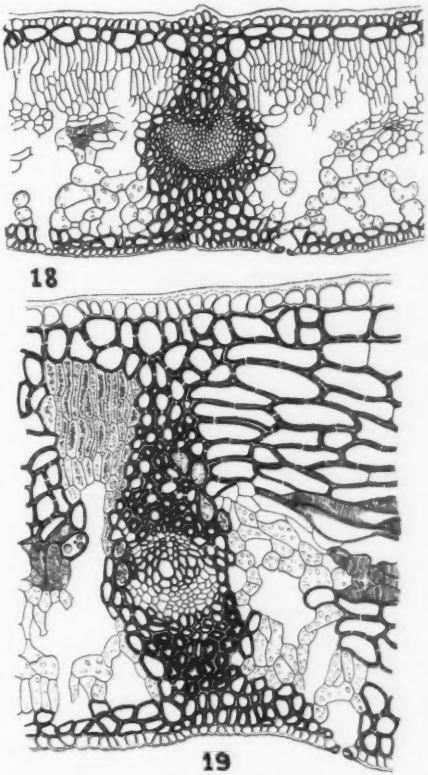


FIGS. 14–17.—*Nothofagus* (BRASS and VERSTEEGH 11115) sp. nov., Netherlands New Guinea. *Fig. 14*, foliage and fruiting habit; fruiting heads consist of woody bivalved funnel-form cupule which invests three nutlets (two are three-winged; one is two-winged). Lobes of cupule joined almost to rim, each lobe covered externally by three or four flat lobular scales. ×1.3. Fig. 15, staminate inflorescence (immature, with one floret aborting). ×2.6. Fig. 16, young fruiting head, fruits still closely invested by involucral segments. ×2.6. Fig. 17, single nutlet; winged character of fruits not so prominent as in antarctic members of genus.

the adaxial (upper) and the abaxial (lower) surfaces of the leaves, usually in close association with the veins. In *N. obliqua*, as in *N. montagnei*, they occur infrequently and only on the upper sides of the leaves.

In the evergreen species, predominantly found in Australia and New Zealand with the exception of the New Guinea species and the two species in South America, the leaves are usually coriaceous and glabrous or with variable pubescence, the margins either entire or minutely crenulate, and practically all dotted with resinous papillae on either the upper or the lower surfaces, occasionally on both. In *N. cunninghami*, glands are borne only at the margins and on the adaxial faces of the small leathery leaves (figs. 8, 9); in *N. menziesii*, glands are sparingly distributed only on the abaxial surfaces; whereas, the equally small, thick leaves of *N. dombeyi* and those of *N. betuloides* are glandulous punctate on both lower and upper surfaces. With the exception of the Australian species, *N. moorei* Krasser, and the recently discovered beeches of the New Guinea area, the evergreen species are characterized by exceedingly small leaves (e.g., 1.2–1.5 cm. in *N. cliffortioides*, 1.5–2 cm. in *N. betuloides*, and 0.5–1.5 cm. in *N. cunninghami* and *N. menziesii*). The leaves of the larger-leaved species of evergreen beech average 2–4 cm. long, occasionally 8–10 cm. on the younger vegetative shoots, and are sharply serrate in their margins. The thick leathery leaves of the New Guinea beeches range 2.5–5.0 cm. long and 1.4–2.0 cm. wide and are characterized by entire, conspicuously recurved margins, with a distinctive notching of the apical portion (figs. 14, 16).

The histological structure of the leaves of these beeches of the antarctic may be described as markedly xeromorphic in most of the evergreen species and as quite typically mesomorphic in the deciduous species, except for a tendency to lignification of both the epidermal and the hypodermal layers and a prominent development of sclerotic tissues in association with the veins. Among the evergreen forms, possibly as an adaptation to the climatic extremes of their environment, all gradations occur in the degree of cuticularization—from the formation of cuticle layers 0.05–0.1 mm. thick, as in *N. cunninghami* (fig. 18), to the elaborate thickenings in the leaves of



FIGS. 18–19.—Fig. 18, *Nothofagus cunninghami*, transection of midrib portion of foliage leaf, with adjacent tissues. ×100. Fig. 19, *Nothofagus* (BRASS and VERSTEEGH 11115) sp. nov., transection of leaf showing one of smaller lateral veins and adjacent tissues. ×110.

certain of the New Guinea species (fig. 19) and in *N. cliffortioides*.

Along with well-developed cutin layers, these evergreen beeches exhibit different degrees of lignification of the cells of the epidermal, as do also those of the hypodermal, layers. In *N. solandri*, and in certain of the smaller-leaved New Guinea beeches (e.g., 11335 of the BRASS and VERSTEEGH collection), there is no marked thickening of either epidermal or hypodermal cells except in the vicinity of the midrib and lateral veins. In *N. cunninghami* the epidermal and hypodermal cells on both sides of the leaf have thick walls, particularly those of the adaxial side. In certain of the larger-leaved New Guinea beeches (e.g., BRASS and VERSTEEGH 11907*a*, 11115, and 10479), the thick-walled hypodermal region is several cells in depth, on both the adaxial and the abaxial sides, and ribs of sclerenchyma tissue extending from the upper and lower hypodermal layers completely envelop the lateral veins, along with the vascular tissues of the midrib, in heavily sclerotinized sheaths. Masses of irregularly shaped scalariform-perforated elements not uncommonly are to be found in such cases in the parenchyma areas between or immediately adjacent to the sclerenchyma-sheathed veins (figs. 18, 19). It is perhaps significant—certainly noteworthy—that among morphological characters emphasized in the Lyginopterideae (36) have been the marked development of sclerenchyma, in the form both of hypodermal ribs and of sclerotic plates located within the internal tissues of stems and leaves; likewise the conspicuous development of stalked, capitate glands in association with the leaf surfaces.

The palisade parenchyma is typically xeromorphic in the evergreen beeches, generally consisting of two to three subhypodermal layers, the cells of which are extremely small and narrow, many containing abundant resin deposits (fig. 19).

STAMINATE INFLORESCENCE AND FLOWER.—The male flowers, solitary or in groups of two to three on short recurved peduncles, in the monoecious species are borne either toward the base of the season's growth or on branches adjacent to those bearing the pistillate inflorescences. Their calyces are either campanulate or funnel form and multilobed. Solitary staminate florets rather than groups predominate in *N. menziesii*, *N. moorei*, *N. betuloides*, and *N. obliqua*, while in *N. dombeyi* and *N. nitida* the grouped arrangement of male florets appears to be a constant feature. In all other species of the genus, however, including the New Guinea beeches, the staminate flowers may occur either singly or in two- to three-flowered dichasia on the same plant.

It has been the writer's observation that the number of staminate flowers (one, two, or three), as also the stamen number, varying from eight to forty (eight to twelve in the smaller florets and twenty to forty in the larger solitary flowers), are characters hardly to be depended upon in the differentiation of the species. On the other hand, even though variable in size, the general form and structure of the anthers (figs. 3, 5, 9, 11, 15), as also the pollen characters, may be relied upon as distinctive of the genus—they are unlike the anther and pollen characters of other genera of the Fagaceae. In general, the anthers in *Nothofagus* conform to a linear-oblong pattern, are two-celled, and are distinctly apiculate; those of *N. procera* and *N. cunninghami* are shortly oblong, the latter with a distinctive curvature of the apex.

In but one of the several species of *Nothofagus* studied in detail has a tend-

ency to hermaphroditism in floral structure been observed, and that is in *N. cunninghami*, the irregularities of which are described under "Pistillate inflorescence and flower."

PISTILLATE INFLORESCENCE AND FLOWER.—The female flowers also occur in simple cymes—one, two, or three (typically three) in a head—the flower heads being borne on erect short peduncles usually in the upper axils of the season's growth, each flower cluster closely invested by a two-, three-, or four-lobed lamellate involucre and a complement of bracts corresponding to the secondary and tertiary floral units of a compound cyme.

Although the segregation of pistillate and staminate flowers in different inflorescences—the pistillate in the upper axils, the staminate in the lower axils of the season's growth—is the general rule in species of this genus, checked in detail in *N. obliqua*, *N. procera*, *N. antarctica*, *N. menziesii*, and *N. moorei*, a certain amount of variability in arrangement and composition of the flowering clusters has been observed, in particular in the Tasmanian species, *N. cunninghami*. The following inflorescence types have been observed in this form—all in association with the flowering shoots of a single season's growth: (*a*) normal three-flowered pistillate inflorescences; (*b*) normal three-flowered staminate inflorescences; (*c*) three-flowered inflorescences consisting of a center pistillate floret with staminate laterals, the latter on short peduncles; (*d*) three-flowered staminate heads, the center floret abortive; and (*e*) solitary pistillate florets, usually with a loose, scaly involucre and occasionally exhibiting abortive stamens (see fig. 9 for habit illustration of some of these types).

Whatever their position and organization, these small, generally inconspicuous, pistillate cymules are, during their early development, very effectually concealed by the broad, variously modeled, membraneous stipules of the subtending foliage leaf (figs. 4, 6).

A valvate or lobular involucre in association with the pistillate flowers and the nutlets is one of the distinctive features of both *Fagus* and *Nothofagus*, but in its external morphology it is extremely variable in the beeches of the southern hemisphere. Variability is seen in (*a*) the number of involucral valves; (*b*) the size and shape of the valves; (*c*) the character and number of the foliar appendages borne dorsally or marginally by the segments; and (*d*) the size and general character of the glands.[5] With the exception of the South American species *N. pumilio* and the evergreen beeches of New Guinea, the cupules associated with the pistillate flowers in species of *Nothofagus* are of the three- to four-valved type, the valves incompletely inclosing three flowers (one dimerous and two trimerous). The pistillate inflorescences of *N. pumilio* and of the New Guinea representatives, however, are characterized by a bivalved involucre which in *N. pumilio* incloses a

[5] The capitate glands, one of the particularly noteworthy and conspicuous features of the cupules of *Nothofagus*, especially of the antarctic species, are large multicellular structures, which, when not apparent as globular stalked appendages borne at the tips of the pinnatifid involucral scales (e.g., *N. moorei*, *N. menziesii*, *N. cunninghami*, and *N. procera*), are frequently to be disclosed in vertical sections of the pistillate inflorescences as sessile, conelike structures borne in the axils of the involucral leaves (fig. 26*A*) or in the axils of the bracts of the inflorescence or even the stipules.

The stalked variety of gland—bearing a striking resemblance to the "glandular spines" identified with the petioles and cupules of *Lyginopteris* (36)—though variable in size, are frequently massive organs (0.5–2.0 mm.), the stalked portion of which has the morphological features of a modified leaf segment. The capitate glandular portion of this organ (fig. 27*A*) consists of a layer of columnar secreting cells, apparently epidermal in origin, which surrounds a spongy core.

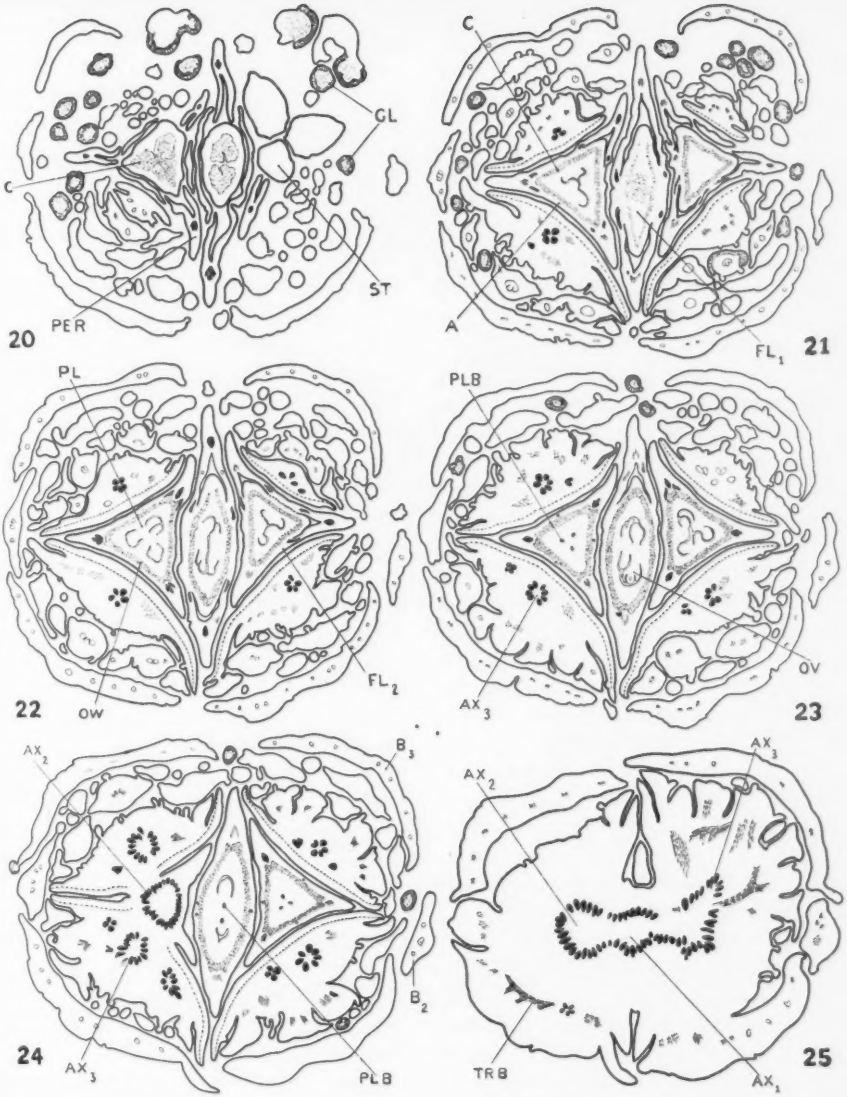
solitary floret and which, in the New Guinea species, incloses either a solitary dimerous floret or a two- to three-flowered group. In the case of the three-flowered groups the flowers may be associated with a single dimerous central floret with two trimerous laterals, or all three may be of the flattened dimerous type, each oriented in the plane of the involucral segments.

Notwithstanding the very noticeable variation in the sizes and shapes of the pistillate florets in the different species of the genus, and the character of their stigmas and conspicuous winged appendages (figs. 1, 6, 7, 8, 10, 13, 17), little variability among the species is apparent in the basic morphology of the ovary, in the origin of the ovules, or in details of vascularization of either carpels or ovules. The individual female flower consists normally of a four- to six-membered perianth (generally four sepals in the dimerous florets and six in the trimerous), borne on the rim of a fleshy and conspicuously angular bi- or tricarpellary ovary, the latter to be interpreted on the basis of ontogenetic evidence as receptacular tissue lined and concrescent with a carpellary core. The outer axial portion of the ovary wall is clearly distinguishable from that which is carpellate in origin, the former being characterized by a loose, somewhat fibrous, texture and the possession of vascular elements, whereas the inner carpellate section is distinguished by a marked zonation which includes an outer *woody* band of variable thickness (figs. 12, 22–25) and an inner fleshy zone. It is from the latter that the three two-ovuled placentae are derived.

Although an axile column of vascularized tissue develops concurrently with the placental tissue and, as in other genera of the Fagaceae (**24, 40**), eventually fuses with it, the relationship of this axile column to the placentae and ovules is not the same as that reported in *Fagus* and *Quercus* (**24**) or in *Castanopsis* (**6**), in all three of which the floral axis produces a thick columnar structure—well supplied with vascular tissue—at the tip of which ovule development is initiated.

VASCULAR ORGANIZATION OF PISTILLATE INFLORESCENCE.—The manner of distribution of the vascular tissues to the female flowers, and to the associated involucre and complex of bracts, is interesting and sheds considerable light on the highly controversial question of the morphological nature of the special involucre in the Fagaceae. A number of hypotheses, based upon studies of *Quercus*, *Fagus*, *Castanea*, and *Castanopsis*, have been proposed in explanation of this structure. Some emphasize the bracteolate character of the cupule (**16**), while others accentuate its essentially axial nature, either as an intercalary formation of the inflorescence appearing between the flower and its bracteoles (**29**) or as the product of the fused secondary or tertiary branches of a dichasium (**13, 26**).

In the vascular organization of the typical pistillate inflorescences in *Nothofagus* (i.e., three-flowered with a four-lobate involucre, well illustrated in *N. procera* and in *N. antarctica*), the three female flowers, one median and two laterals, receive their vascular supply, respectively, from the primary and the secondary vascular axes of the cymule. The vascular cylinder of each floret consists of fifteen to eighteen bundles, some distributed along the sides of the angular floral axis, with others closely associated at the ridged corners of the axis. Generally from the corner units of the floral vascular axis, strands to the central column of the ovary diverge at the base of the flower. Within the axile column of the lateral tricarpellate flowers these



FIGS. 20–25.—*Nothofagus procera*, successive transections of pistillate inflorescence at intervals from apical to basal portions. ×20. (*A*, axial part of ovary wall; AX_1, vascular axis of primary flower of inflorescence; AX_2, vascular axis of secondary floret; AX_3, vascular axis of modified tertiary branch; B_2, secondary bract; B_3, tertiary bract; *C*, carpellate section of ovary wall; FL_1, primary floret; FL_2, secondary floret; *GL*, gland; *OV*, ovule; *OW*, ovary wall; *PER*, perianth; *PL*, placenta; *PLB*, vascular supply to placentae; *ST*, stigma lobe; *TRB*, traces of tertiary bract.)

bundles form a three-strand converging vascular unit which eventually resolves itself into the double bundles supplying the funicles of the six ovules (figs. 23, 24). In the flattened median or primary floret of the typical three-flowered pistillate inflorescence, only two vascular units are observed in the placental axis (fig. 24). This two- or three-strand vascular supply of the axile column of the pistillate florets of *Nothofagus* may perhaps be compared with the inner placental bundles reported in *Castanopsis* (**6**). The prominent cylinder of vascular tissue associated with the axile column of the ovaries of the Castaneae, and with that of *Fagus* and *Quercus*, has not been detected in either the dimerous or the trimerous florets of *Nothofagus*.

The vascular elements to the inner perianth leaves and wings, and likewise those to the styles, are seen to proceed, as conjoint bundles, from the corner units of the floral vascular axis through the marginal (axial) part of the ovary wall to the point of their divergence either to wing, perianth, or stylar members. The rather weak vascular supply to the outer perianth leaves appears to be derived from certain of the units of the floral vascular axis which alternate with those supplying the inner sepals and styles.

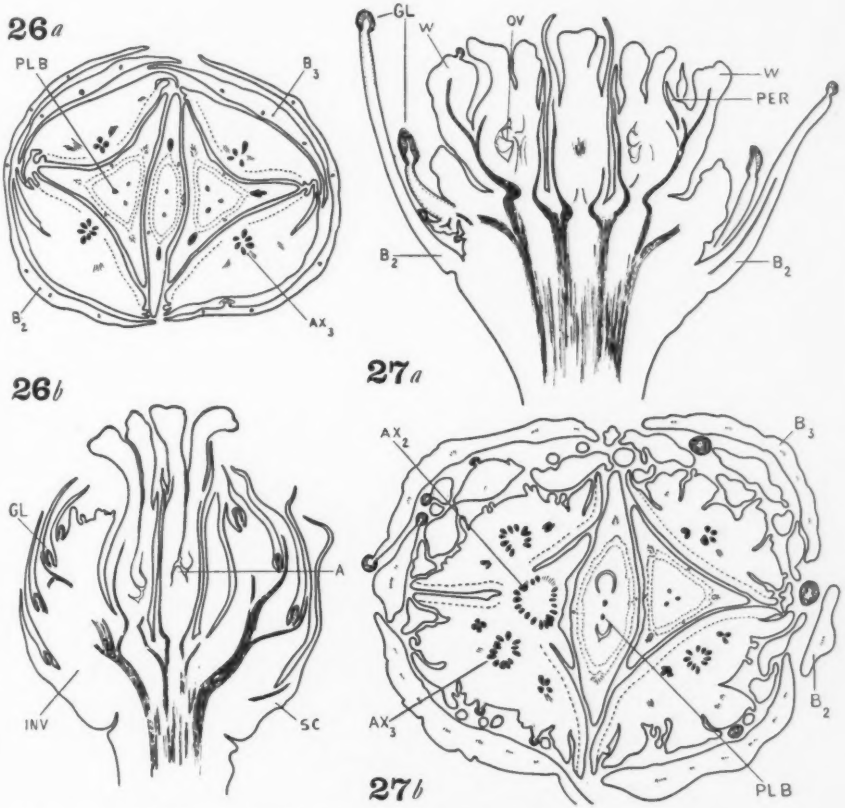
The involucral segments, extending to either side and at right angles to the lateral or secondary floral units of the dichasium, are largely axial in nature; each wedge-shaped valve bears in acropetal succession several to many either lobed or pinnatifid appendages, usually conspicuously glandular (figs. 26, 27). One of the distinctive features of the fruiting heads of the antarctic species of *Nothofagus* is the markedly woody nature of the inner section of the cupule valves, where they adjoin the nutlets (fig. 12), a feature not observed in the more specialized New Guinea species (figs. 28–33, 34–37) or as one of the involucral characters of *Fagus* (**24**). The numerous vascular bundles of each segment of a four-valved involucre will be seen to converge within the axial section as three groups, the center one of each group forming a well-defined, several-strand vascular cylinder, as in the vascular axes of the pedicels of the inflorescence. These "tertiary" pedicellar vascular units in conjunction with those of the secondary axes form a vascular cylinder oriented transversely to that of the combined primary and secondary axes of the inflorescence. The relationship between the vascular units of the involucral segments and those of the secondary axes of the inflorescence is clearly indicated in figures 20–25, depicting successive transections of the triflorous pistillate inflorescence of *N. procera*.

The vascular supply to each of the four tertiary bracts is, like that of the modified tertiary axes, derived from the vascular cylinders of the secondary axes of the dichasium, whereas that of the secondary bracts is derived from the vascular cylinder of the primary axis of the cymule.

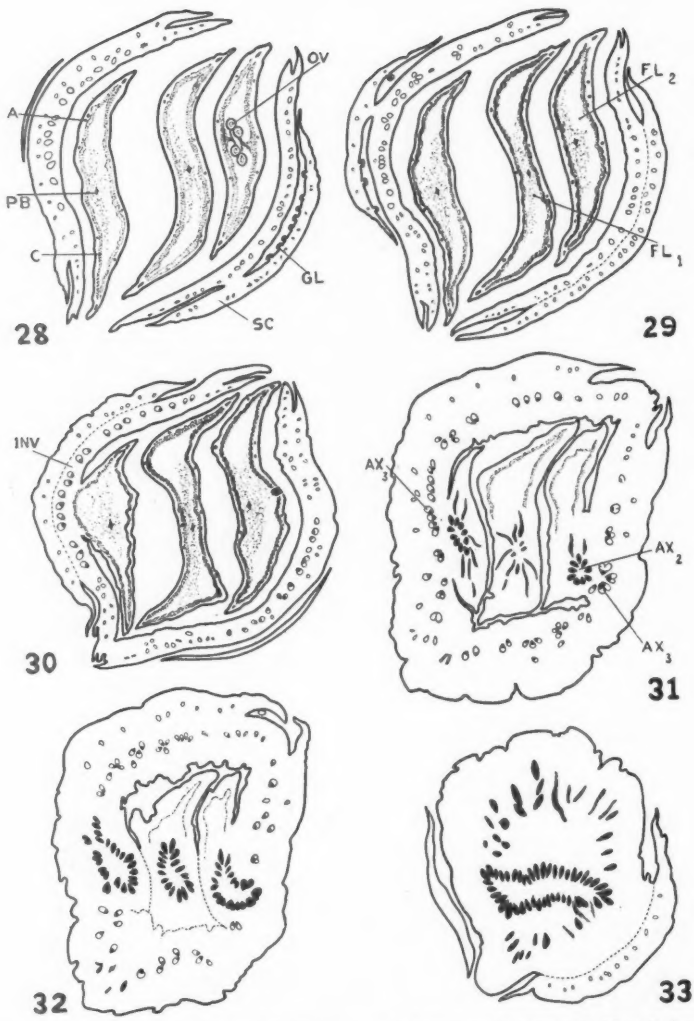
The vascular strands to the secondary bracts are distinct in every way from those directed to the lamina of the subtending foliage leaf and its stipules. Serial transverse sections made to the very base of the primary axis of the inflorescence reveal the vascular systems of each of the rather prominent stipules as very definitely related to the lateral components of the trilacunar trace system supplying the lamina of the foliage leaf which subtends the pistillate flowering group. This is very nearly in agreement with ABBE's interpretation of the vascularization of the secondary bracts and with that of the reduced foliage leaf sub-

tending the pistillate cymules in certain of the Betulaceae (1).

On the basis of vascular organization, therefore, the four bracteolate involucral valves of *Nothofagus* may be interpreted as corresponding to the bracts and modified floral parts, including the axial portions, of the "tertiary units" of an originally more complex cymose inflorescence. This idea has been suggested in a previous report by the writer (25) and has also been proposed, but not very convincingly supported, by BERRIDGE in her studies of the floral morphology of *Castanopsis chrysophylla* (6), in which she asserted that all evidence brought forward in previous work on *Fagus* and *Castanea*, as well as her own studies of *Castanopsis*, are applicable in support of the theory that the valves of the cupule of the Fagaceae are modified secondary or tertiary axes of a dichasial inflorescence and



FIGS. 26–27.—Fig. 26*A*, *B*, *Nothofagus antarctica*, transverse and median-vertical sections of pistillate inflorescence. ×17.5. Fig. 27*A*, *B*, *Nothofagus procera*, median-vertical and transverse sections of pistillate inflorescence. ×15. (*A*, axile column; AX_2, vascular cylinder of secondary axis; AX_3, vascular axis of modified tertiary branch; B_2, secondary bract; B_3, tertiary bract; *GL*, glands; *INV*, involucre; *OV*, ovule; *PER*, perianth; *PLB*, placental bundles; *SC*, involucral scale; *W*, winged extension of ovary wall. Dotted lines indicate areas of ovary wall and involucral valves which undergo sclerotinization during maturation of fruits.)



FIGS. 28–33.—*Nothofagus* (BRASS and VERSTEEGH 11363) sp. nov., Netherlands New Guinea, successive transections of triflorous pistillate inflorescence characteristic of species, all florets of which are flattened and bicarpellate. ×6. (*A*, axial part of ovary wall; AX_2, vascular axis of secondary floret; AX_3, displaced bundles representing vascular units of modified tertiary axes; *C*, carpellate section of ovary wall; FL_1, primary floret; FL_2, secondary floret; *INV*, one of segments of bivalvate involucre; *GL*, gland; *OV*, ovule; *PB*, placental bundles; *SC*, involucral leaves.)

not, as EICHLER (**16**) maintained, merely bracts covered with emergences.

Further evidence in support of this hypothesis is supplied by the single-flowered, and the reduced three-flowered, pistillate inflorescences distinguishing the New Guinea species of *Nothofagus*, although the interpretation of the cupule morphology of these beeches would indeed be difficult without some understanding of the inflorescence morphology of less specialized members of the genus.

COMPARISON OF NEW GUINEA SPECIES WITH OTHER SPECIES OF NOTHOFAGUS

Taxonomic studies dealing with the beeches of the southern hemisphere (**17, 28, 32**) have been in general agreement in emphasizing leaf and involucral characters in keys to the genus, and on the basis especially of foliage characters the sixteen recognized species have been referred to one of three natural sections:

I. Leaves *deciduous*, soft in texture and folded in the bud along the lateral nerves, margins either crenate or serrate; the species in this section, with the exception of *N. pumilio*, all distinguished by four-merous involucres generally with dorsal glands. (1) *N. antarctica* Oerst.; (2) *N. montagnei* Reiche; (3) *N. pumilio* Krasser; (4) *N. obliqua* Blume; (5) *N. procera* Oerst.; (6) *N. gunnii* Oerst.

II. Leaves *evergreen*, usually very coriaceous and glabrous, or with only slight pubescence, margins coarsely or finely serrate or crenate, the surfaces of the leaves—either upper, lower, or both—conspicuously dotted with resinous papillae; the species of this section also characterized by four-merous involucres, the segments generally with leafy pinnatifid appendages with stalked glands. (7) *N. apiculata* Krasser; (8) *N. menziesii* Oerst.; (9) *N. cunninghami* Oerst.; (10) *N. betuloides* Blume; (11) *N. dombeyi* Blume; (12) *N. nitida* Krasser; (13) *N. fusca* Oerst.; (14) *N. moorei* Krasser.

III. Leaves *evergreen* with entire margins, the leaves on young trees generally glabrous, those on older trees densely tomentose; the two species in this section, (15) *N. cliffortioides* Oerst. and (16) *N. solandri* Oerst., both distinguished by three-merous involucres.

It is with the third section of the three that the New Guinea beeches appear to be most clearly allied, although they exhibit characteristics sufficiently distinctive in their leaves, cupules, and flowers to warrant their recognition either as a subsection of III or as an independent fourth section of the genus.

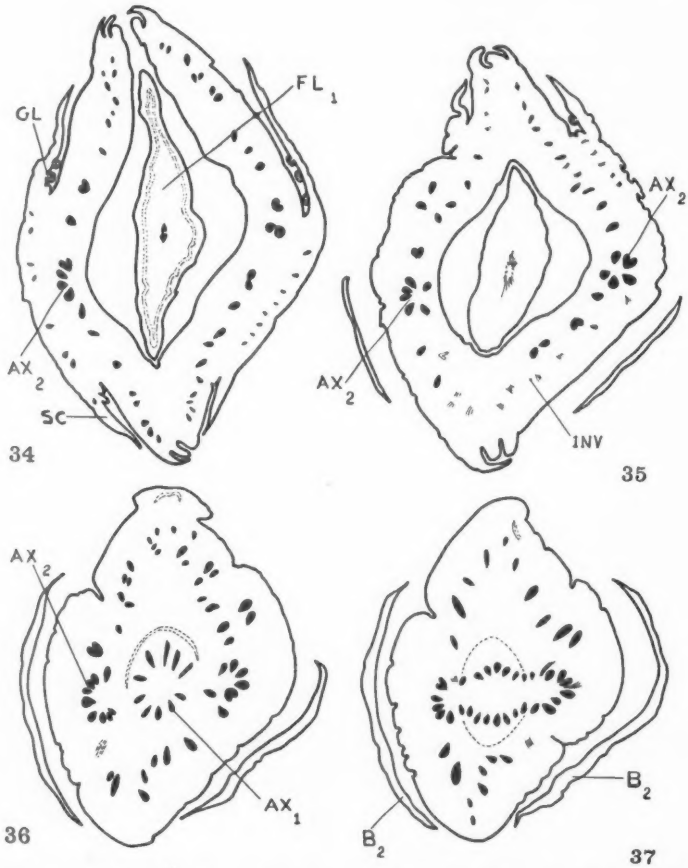
With one exception (i.e., BRASS and VERSTEEGH 11335), the five types of New Guinea beeches examined by the writer are characterized by *evergreen* leaves, thick and coriaceous in texture, their upper surfaces glabrous, the lower somewhat tomentose and conspicuously dotted with resinous papillae, margins entire and recurved with a distinctive notching of the apical section. The sizes are variable, ± 3.5–4.5 cm. in length by ± 1.5–2.0 cm. in breadth, but generally are larger than those of the antarctic members of the genus. As in other species of *Nothofagus* broad, scalelike stipules, peltate in shape and glandular at the base, are a conspicuous feature of the younger shoots, partially investing the young foliage leaves and the flower buds.

The anatomical features of the leaves of these beeches have already been described and compared with those of other evergreen species in the genus. Summarized, they include: (*a*) heavy cuticularization; (*b*) lignification of the cells of both the epidermal and the hypodermal layers, the latter frequently several layers in extent; (*c*) grouping of hypodermal cells into ribs in association with the vascular tissues; and (*d*) occurrence in the larger-leaved species of scalariform perforated transfusion elements bordering, or in the immediate vicinity of, the sclerenchyma-sheathed veins (fig. 19).

The four or five distinct species represented in this New Guinea collection are all characterized by a thick, scaly bi-

valvate cupule, the segments of which may be free almost to the base of the fruiting head or joined almost to the rim of the spreading valves (fig. 14). This funnel-formed cupule may inclose either a solitary flattened and two-winged nutlet (e.g., BRASS and VERSTEEGH 12673, 11333), illustrated in section in figures 34–37, or a group of three, in which the nutlets are associated, two trigonous and three-winged with one flattened and two-winged (e.g., BRASS and VERSTEEGH 11115, 11369), or all three may be of the flattened two-winged type and all oriented diagonally to the involucral lobes (e.g., BRASS and VERSTEEGH 11363), illustrated in section in figures 28–33.

In their bilobed involucres and predominantly bicarpellate dimerous florets, the latter frequently reduced to a solitary flower per cymule, the New Guinea beeches exhibit greater evidence of specialization than is apparent in the more typical members of the genus, i.e.,



FIGS. 34–37.—*Nothofagus* (BRASS and VERSTEEGH 11335) sp. nov., Netherlands New Guinea, successive transections of uniflorous pistillate inflorescence characteristic of species. ×13. AX_1, vascular cylinder of primary floral axis; AX_2, vascular units of modified secondary axes; B_2, secondary bract; FL_1, primary floret of cymule; *GL*, gland; *INV*, one of segments of bilobed cupule; *SC*, involucral leaves.)

those native to antarctic South America and to the Australian–New Zealand area. The latter, in their predominantly small evergreen leaves and tricarpellate florets —the pistillate flowers and the fruits associated usually in groups of three within a woody and conspicuously glandular four-valved involucre—exhibit a combination of characters which, in the opinion of the writer, is the most primitive to be found in the existing members of the beech series, if not in the entire Fagacean assemblage.

The morphological features of the flattened dimerous floral types, predominating in the New Guinea beeches, are about as we find them in the median floret of the more typical triflorous inflorescences in the genus (figs. 20–25). The ovary wall is thinner, however, and lacks the woody tissue characterizing other species of *Nothofagus;* likewise, there is a noticeable absence of sclerotic tissue in the thick involucral valves. The multicellular resin-secreting glands, borne dorsally by the involucral leaves of most of the beeches of the southern hemisphere (figs. 1, 8, 10), are in the New Guinea species borne ventrally by the involucral scales (figs. 29, 34).

In the triflorous pistillate inflorescences the vascular supply of each of the broad semicircular involucral valves (figs. 28–33) is seen to consist of a rather loosely organized core of vascular tissue and, extending to either side of this, of numerous bundles which spread fanlike from the point of their divergence from the vascular cylinders of the secondary axes of the dichasium. These bundles, reinforced by the traces of the involucral leaves, may be interpreted as the displaced vascular elements of the highly modified tertiary branches of the inflorescence. Likewise, on the basis of vascular organization, it seems evident that the involucral valves of the single-flowered cymules in this group (figs. 34–37) bear the same relationship to the primary axis of the inflorescence that the secondary axes bear in the more typical three-flowered cymules. There can be little doubt, moreover, that each of the cupule segments of the bivalved uniflorous species of *Nothofagus* represents one of the greatly modified secondary axes of a dichasial inflorescence. In the New Guinea species of *Nothofagus*, reduction and general modification of an originally more complex dichasial inflorescence have extended not only to tertiary but in certain cases even to secondary units, the florets of which have either been lost or greatly modified in the process of reduction of the inflorescence.

The vascularization of the pistillate flowers, as does the manner of origin of placentae and ovules, follows the same basic plan as that of other species of the genus.

Summary

1. Anatomical features, not hitherto reported, found to characterize the foliar organs, expecially of the evergreen species of the genus *Nothofagus*, include: (*a*) heavy cuticularization; (*b*) lignification of the cells of both the epidermal and the hypodermal tissues, the latter occasionally several layers in extent; (*c*) grouping of hypodermal cells into ribs in association with the vascular tissues; and (*d*) occurrence in the larger-leaved species of scalariform-perforated transfusion elements bordering, or in the immediate vicinity of, the sclerenchyma-sheathed veins.

2. Internal morphological characters in the flowers and fruits, which have been determined to be sufficiently constant in nature to supplement external morphological characters in a definition of this genus, include: (*a*) the occurrence of generally heavy, though variable, amounts of sclerotinization both of the ovary wall

and of the cupular valves; (*b*) a distinctive type of origin and vascularization of the placentae; (*c*) the occurrence of distinctive multicellular resin-secreting glands in association with either the dorsal or the ventral surfaces of the involucral scales, the cupules of certain of the species—in their peculiarities of glandular structure—bearing a striking resemblance to the gland-bearing husks enveloping the seeds of the Lyginopterideae.

3. The anatomy and morphology of the flowers and inflorescences of species of this genus furnish evidence in support of (*a*) the origin of the basic triflorous inflorescence characterizing the Fageae from an ancestral many-flowered dichasium, the individual florets of this hypothetical cyme being trimerous and probably bisporangiate, and of (*b*) the evolution of the lobular type of cupule in association with the pistillate florets as the product of reduction and general modification, including fusion, of sections of this originally more complex cymose inflorescence, such modification accomplishing greater protection for a few of the more advantageously located florets and fruits.

4. The beeches from Netherlands New Guinea, concerning which there has been some question as to position in the Fagacean series, have been found to exhibit a combination of morphological characters in their foliage and reproductive structures which is distinctly that of *Nothofagus*, although in certain respects they are sufficiently unlike the more typical members of that genus to justify their reference to a new section of the evergreen beeches.

Grateful acknowledgment is made to Dr. ELMER D. MERRILL, Arnold Professor of Botany, Harvard University, for the generous loan of herbarium materials as well as for valuable suggestions in the progress of this work. The writer is also indebted to Dr. A. C. COTTON, Keeper of the Herbarium and Library, and to Dr. C. A. METCALFE, Keeper of the Jodrell Laboratory at Kew Gardens, London, for fixations of flowering specimens of *Nothofagus* species; likewise to the Regius Keeper of the Royal Botanic Garden of Edinburgh.

DEPARTMENT OF BIOLOGY
GOUCHER COLLEGE
BALTIMORE, MARYLAND

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MORPHOLOGY OF SPORE FORMS AND HETEROECISM OF THE GIANT BAMBOO RUST, DASTURELLA DIVINA

M. J. THIRUMALACHAR, M. J. NARASIMHAN, AND K. S. GOPALAKRISHNAN

Introduction

Leaf rust on giant bamboo (*Dendrocalamus strictus* Nees) has recently been decribed by MUNDKUR and KHESWALLA (**6**) as belonging to a new genus *Dasturella* and as a member of the tribe Raveneliae of DIETEL (**4**). It had previously been described by SYDOW (**7**) as *Angiospora divina*, based on collections made by Dr. R. N. TANDON at Majhgawan, India, the host being tentatively identified as *Bambusa* sp. In a re-examination of the material, MUNDKUR and KHESWALLA found that the telial heads, instead of being nonerumpent and lenticular as in the genus *Angiospora* Mains, were distinctly erumpent and formed flabelliform crusts. As the rust was thus distinct from any species of *Angiospora*, they proposed the new genus *Dasturella*

for its accommodation. The host was reidentified as *Dendrocalamus strictus*. *Dasturella divina*, which is the type of the genus, has been recorded from Tonkin and Japan.

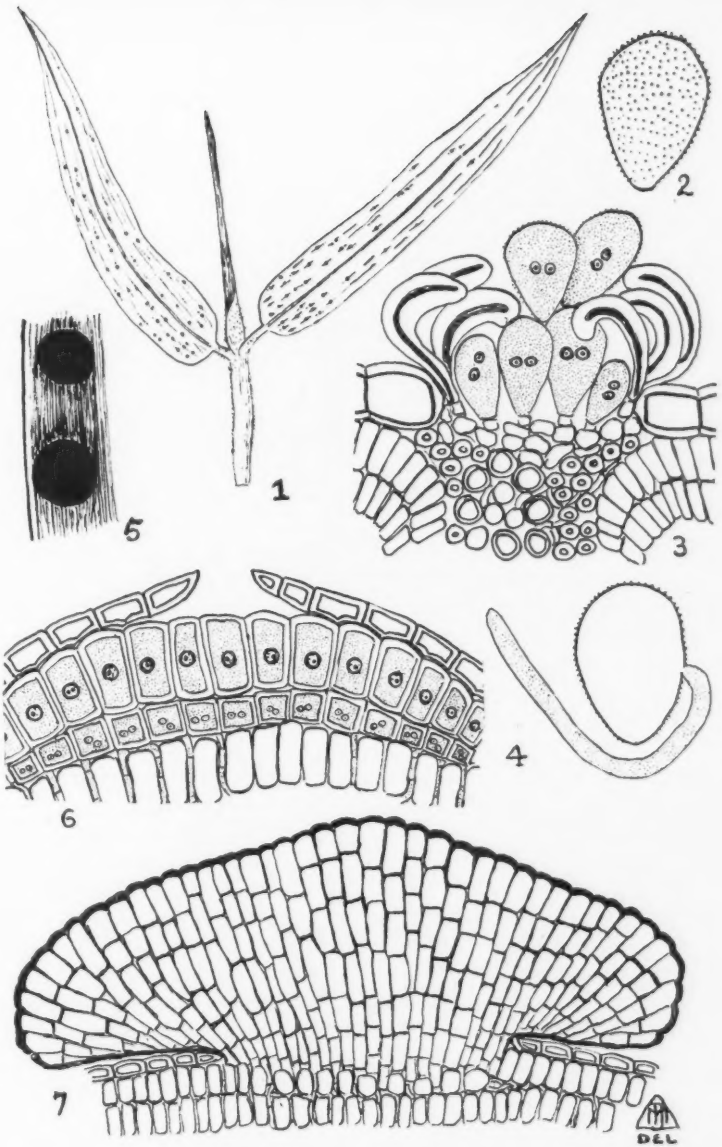
In the course of studies on the rust flora of South India, the authors encountered large tracts of forests in Mysore with the giant bamboo being heavily parasitized by a leaf rust. The rust was found to be *Dasturella divina*, and the identification was checked by comparing it with the type from Herbarium Cryptogamae Indiae Orientalis, New Delhi. Parts of the districts of Kadur and Shimogga in Mysore have large areas covered with the giant bamboo, and a brief survey of these areas indicated the presence of the rust over a wide range. In the present investigation the writers have been successful in establishing the heteroecious nature of the rust by a series of field studies and inoculation experiments. The morphology of the spore forms has been studied with a view to adding more information on the diagnostic characters of the genus and species.

LIFE-HISTORY AND HOSTS.—*Dasturella divina* and *D. bambusina* Mundkur & Khes. were described as hemi forms, the pycnial and aecial stages being unknown. *D. divina* which occurs in Mysore is foliicolous. The uredial stages of the rust develop in abundance on the leaves of the giant bamboo soon after the commencement of the southwest monsoons. The infection spots are evident as minute linear specks on the lower sides of the leaves. The sori are erumpent, striate, and coalesce with one another (fig. 1).

The uredia appear macroscopically as rusty-brown striate sori throwing up large masses of yellowish-brown spores. The rust increases in quantity, as the season advances, through the production of secondary uredia. About the beginning of October, or sometimes as late as November, the telia are first noticed. They are developed at first within old uredia but later are formed independently. The telia are macroscopically black, arising singly or in groups, and appear as flattened pinheads in surface view (fig. 5).

Since pycnial and aecial stages were so far unknown for the genus *Dasturella*, an attempt was made, in surveying the large areas of bamboo forest affected by *D. divina*, to find these stages. Since they are not present on bamboo, attention was focused on a number of unconnected aecia occurring on various hosts in the jungle. The possible oversummering of the rust in the uredial stage was not studied in detail. Since the bamboo shoots remain defoliated for long periods, at which time no viable urediospore inoculum could be collected, chances of the rust perennating in the uredial stage appeared to be slight.

During a number of excursions made by the senior author in the bamboo forests of Mysore, there was noticed a profusion of certain plants bearing aecial stages in consonance with the abundance of giant bamboo bearing uredial and telial stages. In a survey of the locality between Sacrabyle and Megaravalli in Shimogga district, Mysore, he observed the occurrence of an aecial stage in great abundance on *Randia dumetorum* Lam., a member of the Rubiaceae. It was always associated with the giant bamboo rust in the same locality. Only pycnial and aecial stages were present on *Randia*. The infections produced considerable malformations. Pycnial and aecial stages on this host appeared in May and June and always preceded the appearance of the rust on giant bamboo following southwest monsoons. Plants of *R. dumetorum* which



FIGS. 1–7.—Fig. 1, infected leaves of *Dendrocalamus strictus*. Natural size. Fig. 2, urediospore. ×1000. Fig. 3, section through uredium. ×500. Fig. 4, germinating urediospore. ×1000. Fig. 5, surface view of telium. ×80. Fig. 6, section through young telium. ×500. Fig. 7, mature telium. ×250.

grew in places where the giant bamboo did not show rust were all free from this type of infection. This continued association of the rust on *Randia* with the giant bamboo rust led the senior author to suspect that they might be causally connected. Inoculation experiments confirmed this surmise and have established the fact that *D. divina* is a heteroecious eu-form with uredia and telia on the giant bamboo and pycnia and aecia on *R. dumetorum*.

Material and methods

Material for morphological studies of the spore forms were fixed in formalin–acetic-alcohol and in Karpechenko's modification of Navashin's fluid. Microtome sections 8–10 μ thick were stained by Newton's gentian-violet method, with light green as counterstain. The aeciospores, urediospores, and a few of the teliospores were germinated and stained by the method suggested by the senior author (10). Inoculation experiments with urediospores and aeciospores were carried out following the usual procedures. Pieces of leaves bearing mature and freshly erupted uredia and aecia were rubbed on leaves of the plant to be inoculated after first moistening the latter with a spray of water. The inoculated plants were inclosed in moist chambers for 48 hours, after which they were transferred to the greenhouse for observation. The procedure suggested by CLINTON and MCCORMICK (3) for rust infection experiments in Petri dishes was carried out in a number of cases, especially in conducting aeciospore inoculations of bamboo leaves. This method reduces the possibility of contamination from airborne spores, but its success usually depends upon the length of time over which excised leaves are able to retain their fresh state in moist chambers. The bamboo leaves remained fresh for nearly $1\frac{1}{2}$ months—long enough for successful inoculations to be confirmed.

Observations

Life-cycle on giant bamboo

UREDIA.—Some of the morphological details of the spore forms of uredia and telia were given by MUNDKUR and KHESWALLA (6). Uredia are developed in June–July. The sori are hypophyllous, minute, and striate. Sections through the uredium indicate that the sorus is subepidermal (fig. 3), becoming erumpent by the rupture of the epidermis. The sorus is lined along the margin by a fringe of cylindric, deeply incurved paraphyses which inarch over it. The paraphyses are hyaline to pale yellow, rounded at the apex, and possess a central core. The urediospores are sessile, cinnamon yellow, rounded at the apex, obovate to ellipsoid, and measure 17–25×15–24 μ. The germ pores are distinct, and the epispore is covered with dense echinulations (fig. 2).

The urediospores germinate readily when placed in moist chambers, and germ pores become clearly visible as the germ tubes emerge (fig. 4). The two nuclei and the cytoplasmic contents of the spore migrate into the germ tube. The urediospores readily infect the healthy leaves of giant bamboo, causing secondary infections, and are mainly responsible for the spread of the disease.

TELIA.—Telia follow uredia in development in October, appearing as tiny black specks, and can be differentiated with the aid of a hand lens from infection spots of *Phyllachora* sp. which sometimes occur on the same leaves. They arise either singly or in groups. Stages in the development of telia are as follows: From the mass of hyphae com-

posing the basal hymenium beneath the epidermis, a single row of one-celled teliospores is first differentiated. They become laterally coalescent to form a *Melampsora*-like crust (fig. 6). More spores begin to be differentiated from the base, resulting in the production of chains of teliospores. The spores so produced do not become separated from one another but remain fused both laterally and at either end to form a flabelliform crustose head (fig. 7). The epidermis is ruptured and the telial column becomes exposed. No paraphyses are associated with the telia that are developed independently, but in those cases in which telia are formed within old uredia one can observe the marginal fringe of paraphyses. A thin hyphal layer envelops the entire telial head.

Cytological studies indicate that young teliospores are binucleate but that in mature spores the two nuclei fuse (fig. 6). The mature spores are rectangular to cuboid, yellowish brown, smooth, and measure 12.5–30×10–16 μ, with a mean of 20×12.5 μ. The topmost spores of each chain possess apical thickenings which fuse with one another to impart additional firmness to the telial head. MUNDKUR and KHESWALLA studied the telium from herbarium material and considered that it was composed of fasciculate five- to seven-celled teliospores which had coalesced laterally to form a flabelliform crust. They were led to this conclusion by noticing the plane of cleavage of the spores in the crushed telium. It might, however, be pointed out that if the teliospores are considered to be five- or seven-celled, comparable with the condition present in such genera as *Phragmidium*, etc., then all the cells of a teliospore must have arisen through septations of a single teliospore initial and must all be of the same maturity. The developmental stages of the telium observed in *D. divina* in the present study have indicated that the teliospores are produced in catenations, the oldest spore being situated at the outer end and the youngest one at the base. A similar structure for the telium of *D. grewiae* (Har. & Pat.) Thirumalachar has been indicated by one of us recently (11). The telium should therefore be considered as being composed of one-celled spores. The teliospore measurements given by MUNDKUR and KHESWALLA (especially the length of the spore) refer to the entire teliospore chain and not to individual spores.

Only a few cases of teliospore germination have been observed. The spores were found to require a period of rest (3 months), and in germination behavior they are very capricious. The promycelium is external and four-celled, bearing globular sporidia on short sterigmata. Because of the scarcity of germinating teliospores, sporidial inoculations could not be carried out.

LIFE-CYCLE ON RANDIA DUMETORUM

That the aecial stage on *R. dumetorum* might be causally connected with the rust on giant bamboo was first surmised from field studies and was later confirmed by aeciospore inoculations. Sixty-two excised young leaves of bamboo in thirty-one Petri dishes were inoculated with aeciospores from *Randia* by methods already described. The giant bamboo leaves were collected from healthy culms in localities where there were no traces of the rust, and an equal number of leaves was set apart as controls. The inoculations were made on May 10, 1946, when, because of heavy rains, the atmospheric humidity was as high as 90%. The temperature in the laboratory ranged between 24° C. and 30° C.

The first sign of successful infection appeared on the 17th day after inoculation as minute discolored spots. These gradually changed into olive-yellow striate spots by the 22d day. About the 27th or 28th day the sori had developed, throwing up masses of urediospores. In some cases the time required for the development of urediospores was as much as 30–34 days. Urediospores thus obtained were used in inoculating other healthy leaves of giant bamboo, and a second crop of uredia was produced. The spores were also compared microscopically as to structure and measurements with the collections of *D. divina* occurring naturally on the host and were found to be identical. In none of the controls was any trace of infection discernible, and they remained healthy.

The penetration of the aeciospore germ tubes into the giant bamboo leaves was studied in sections. No detailed studies on the pathological histology were made. Sections of bamboo leaves fixed 3 days after inoculation with aeciospores from *Randia* revealed the penetration through the stomata. The germ tube of the aeciospore forms an appressorium on the stoma (fig. 11) and, after penetration, forms a substomal vesicle. Some of the sections of material fixed 15 days after inoculation showed extensive development of intercellular hyphae and the organization of the uredial initials.

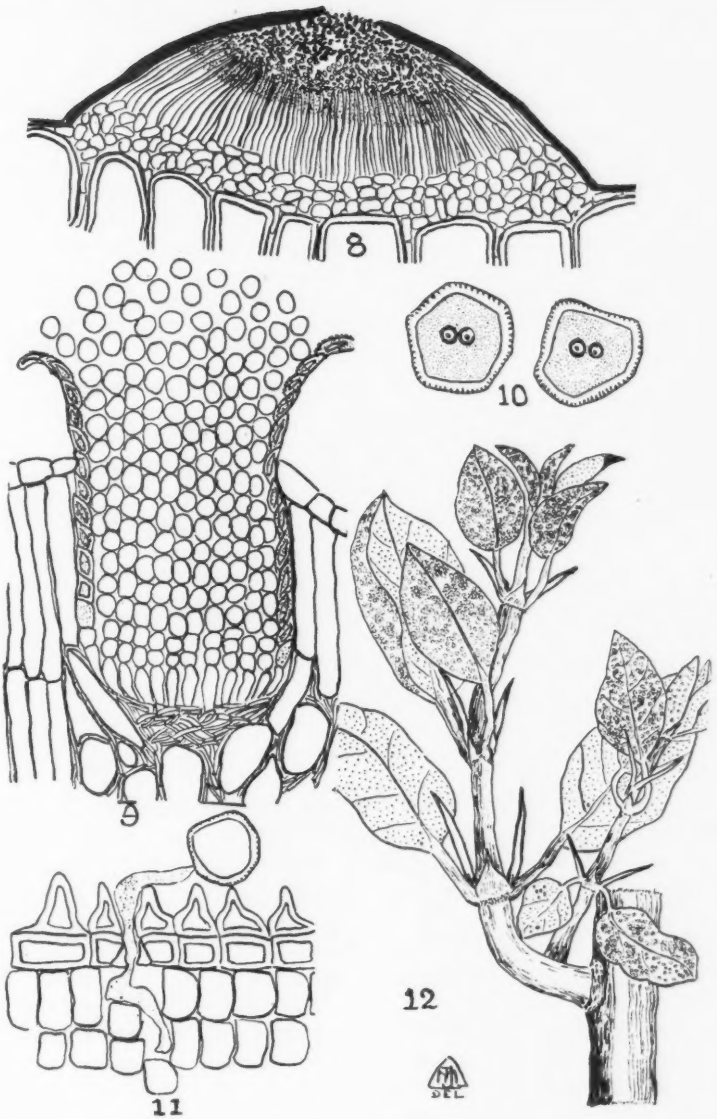
The rust appears on *R. dumetorum* under natural conditions in May and June. At first few leaves are rusted, the infection patches appearing yellow and hypertrophied. Very soon, however, owing to the infection of the meristem in the leaf axils, large witches' brooms showing systemic infection are produced. These witches' brooms have short internodes and a rosette appearance (fig. 12). The bright orange color of the aecia makes the witches' brooms conspicuous in the field.

Pycnia and aecia are developed in abundance on the infected parts. The pycnia are epiphyllous (in the case of leaves, while on the stem they are distributed all over), subcuticular (fig. 8), and appear on the infection patches as tiny orange-yellow specks. They are formed by a uninucleate mycelium and do not possess conspicuous ostiolar paraphyses. The pycniospores are produced in abundance imbedded in nectar.

The aecia are cupulate, hypophyllous (on the leaves), orange-yellow, and possess well-developed peridia (fig. 9). The process of diploidization has not been followed. The binucleate basal cells abstrict numerous spores in succession. Sterile intercalary cells have not so far been observed in the spore chains. The mature aeciospores are subglobose to polygonal, orange-yellow, and show two distinct nuclei in stained preparations (fig. 10). The germ pores are indistinct, and the epispore presents a verruculose appearance because of deep pitting. The spores measure 18–24×15–19 μ. The peridial cells are angular to fusoid, thick-walled, abutting on the sides, smooth on the outer surface and densely rugose on the inner side, and measure 27–40× 15–22 μ.

Discussion

Regarding the identity of the *Aecidium* occurring on the host genus *Randia*, mention may be made of *A. randiae* P. Henn. (5) and of *A. abscendens* Arth. and *A. pulverulentum* Arth. (1). *A. randiae* was described from material collected by ULE in Brazil. The host was cited as *Randia* but was later found to be *Basacantha* (8). *A. abscendens* and *A. pulverulentum* were recorded from Puerto Rico and Mexico, respectively.



FIGS. 8–12.—*Fig. 8, pycnium developed on leaves of Randia dumetorum.* ×800. *Fig. 9,* aecium. ×250. Fig. 10, mature aeciospores. ×750. Fig. 11, section through artificially inoculated leaf of bamboo with germinating aeciospore. ×500. Fig. 12, portion of witches' broom on *Randia dumetorum* showing systemic infection. Natural size.

The former is parasitic on *R. aculeata* and very closely resembles the pycnial and aecial stages of *Dasturella divina* described in the present study. The pycnia in *A. abscendens* (2) were stated to be subcuticular, the aecia being cupulate and peridiate. The measurements of its aeciospores and peridial cells differ slightly from those of *D. divina*. An *Aecidium* on *R. dumetorum* collected near Sirsi, India, was placed under *A. randiae* by UPPAL *et al.* (12). Since the material was not available for comparison, its identity is not certain.

Pycnial and aecial stages having been found in the present study, the genus *Dasturella* might be described as follows:

Dasturella MUNDKUR & KHESWALLA

Pycnia subcuticular, conoid, without conspicuous ostiolar paraphyses. Aecia subepidermal, cupulate, with well-developed peridia. Uredia subepidermal, with marginal incurved paraphyses and sessile urediospores. Telia subepidermal, erumpent, reddish-brown, in flabelliform heads; teliospores one-celled, developed in basipetal catenations, with the spores united both vertically and laterally to form compact fasciculate crusts; spores germinating after a period of rest by a four-celled external promycelium. Type species: *Dasturella divina* (Syd.) Mundkur & Khes.

The genus *Kweilingia* Teng has been described (9) as parasitic on *Bambusa* sp. The telia of this rust were stated to be erumpent, confluent, and crustose, with teliospores occurring in chains of one-celled spores which were laterally united. The rust was first described by TENG as *Chrysomyxa* and later on was accommodated in his new genus *Kweilingia*. This rust is a microcyclic form and is distinctly separate from *Dasturella*.

The genus *Dasturella* was placed by MUNDKUR and KHESWALLA under the tribe Raveneliae of DIETEL (4) on account of its fasciculate sessile telial heads, resembling to some extent those of *Nothoravenelia* Diet., a member of that tribe. In general, however, the members of that tribe possess multicellular telial heads which are not only erumpent but become pulverulent. The presence of cysts in *Nothoravenelia*, characteristic of most of the Raveneliae, would seem to remove the genus *Dasturella* (which lacks them) farther from that tribe. It appears more reasonable to accommodate it in the tribe Ochrosporae of DIETEL's classification of the Pucciniaceae along with *Cerotelium*. The characters enumerated for the Ochrosporae are the presence of subcuticular pycnia, peridiate cupulate aecia, uredia with hyphoid paraphyses or peridium, and telia in crusts, often with catenate spores. All these characters are well represented in *Dasturella*. The telial crusts of *Cerotelium*, although columnar at the base, become pulverulent at the apex, with the spores getting separated by germinating. In *Dasturella*, on the other hand, the teliospores are firmly united to retain their flabelliform shape and are resting spores.

Summary

1. *Dasturella divina* (Syd.) Mundkur & Kheswalla is a heteroecious eu-form with uredia and telia on the giant bamboo (*Dendrocalamus strictus* Nees), and pycnia and aecia on *Randia dumetorum* Lam. (Rubiaceae). The causal connection between the stages of the life-cycle on *Randia* and on the giant bamboo was first surmised from the continuous association of the two rusts in bamboo forests and was later confirmed by inoculation experiments.

2. Infections on *Randia* produce marked hypertrophy and formation of witches' brooms following systemic invasion of the host. The pycnia are subcuticular and conoid, borne on haploid mycelia. The aecia are cupulate and

peridiate, and the aeciospore chains lack sterile intercalary cells.

3. *Uredia and telia occur on the leaves* of giant bamboo. The sori are striate, and morphological details of these two spore forms have been given by MUNDKUR and KHESWALLA. The telia, which occur *in flabelliform erumpent crusts*, are, however, contrary to their description, composed of one-celled, catenate spores produced in basipetal succession. The accommodation of the genus in the tribe Ochrosporae of DIETEL is suggested. Aeciospores from *Randia* were found to infect the leaves of the giant bamboo, inciting the rust on the bamboo. The period of incubation ranged from *27 to 34 days*. The urediospores spread the disease by secondary infection.

MALLESWARAM, BANGALORE, INDIA

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GROWTH AND MORPHOLOGY OF TOBACCO TISSUE CULTURES IN VITRO

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 580

SAMUEL M. CAPLIN

Introduction

A milestone in botanical research was reached in 1939, when WHITE (**22**), using stem tissue from tobacco, and GAUTHERET (**4**) and NOBECOURT (**13**), using cambial tissue from carrot root, showed independently that excised plant tissues will grow indefinitely if they are repeatedly subcultured to fresh nutrient of the proper constitution. After HABERLANDT (**8**) had formulated the idea of a plant tissue culture at the beginning of the century, the first big step was made when ROBBINS (**15, 16, 17**) and KOTTE (**11, 12**) attempted independently to grow excised root tips and stem tips in nutrient solutions of various composition. By repeated subculture ROBBINS (**16**) obtained a decreasing growth rate for root tips which suggested that unknown material not present in the nutrient might be essential for continued growth. Although GAUTHERET (**7**) learned much about nutrient requirements by attempting to grow cambium from large trees, WHITE (**18, 20, 21**)

measured growth of excised root tips and used new information from nutrition and biochemistry to develop a synthetic nutrient for the continued growth of excised roots. This done, he was able to culture excised stem tissues (**22**). On the surface of an agar medium the latter grew in an amorphous, undifferentiated state, and by periodic subculture to fresh nutrient they were shown to possess an unlimited capacity for growth. Detailed historical reviews have been adequately given by WHITE (**19, 23, 24**).

Further investigation of the conditions necessary for growth of plant tissue cultures was undertaken by HILDEBRANDT *et al.* (**9, 10**). In order to compare various treatments, they cut cultures into pieces approximately 20–30 mg. in weight, allowed them to grow for 6-week periods, and determined the final fresh weights. Through the use of sufficient tissues and replications and statistical analysis of the data, they were able to work out the optimal temperatures, pH, and sucrose concentrations (**9**) and the optimal concentrations of the other constituents of a nutrient for tobacco and of one for sunflower callus cultures (**10**). Their methods and results were necessary groundwork to further research involving the culture of excised plant tissues.

Thus far work by various investigators has resulted in (*a*) obtaining plant tissues which will grow in culture and (*b*) determining the optimal conditions for their growth. Now that these problems have been partially solved, more attention can be directed to investigation of the nature of the growth process itself in cultures of excised plant tissue. The experiment described here concerns the mechanism of increase in size of a tobacco callus culture and its rate of growth.

Material and methods

The tissues used were 6-week-old subcultures generously supplied by Dr. A. C. HILDEBRANDT from a stock culture which he obtained in 1941 from Dr. P. R. WHITE. WHITE has described (**18**) the isolation of the original cultures from fragments excised from a region about 5 cm. from the stem tip of *Nicotiana glauca* Grah. ♀ × *N. langsdorffii* Weinm. ♂. Of the original explants, only those which actually contained cambial tissue continued to grow.

The nutrient used was the modification by HILDEBRANDT *et al.* (**10**) of WHITE's semisolid medium for tobacco callus tissue, the composition of which was kindly imparted by Dr. HILDEBRANDT prior to publication of their report. Salts and sucrose were reagent grade, and redistilled water was used in preparing the nutrient. Difco brand "Bacto Agar" was used at 0.5% concentration. Experimental cultures were grown in the dark in 125-ml. Erlenmeyer flasks containing 50 ml. of medium. For the first 3 weeks they were in an incubator at 26° ± 1.0° C. and were then transferred, because of approaching summer, to a thermostatically controlled refrigeration unit equipped with a heater operating at this temperature.

Tissues were cut into uniform cubes by means of a dicer consisting of a set of razor blades separated by flat washers carefully measured with a micrometer caliper and held together with small bolts. Two such instruments were made; one had a distance between blades of 1.7 mm., and the other of 2.7 mm. For practice a block of potato was cut into slices, the slices into shoestrings, and the shoestrings into cubes. The weights to the nearest 0.1 mg. of the first thirty cubes of potato picked at random from

each group are shown in table 1. The lesser variability of the larger cubes can be ascribed to variation in the distance between blades during the cutting process; these variations were relatively greater in the smaller cubes.

Experimental

Fifteen tobacco cultures 10–15 mm. in diameter were cut into cubes with dicer A, and ten cultures with dicer B; within each size group all cubes were mixed. Each culture flask was then inoculated with four cubes and was placed in the culture chamber. After contaminated cultures had been eliminated, 168 cubes remained in group A, 71 in group B. At weekly intervals four or five culture flasks in each group were removed; the cultures were rinsed in water, drained on moist filter paper in a covered Petri dish in order to prevent drying, weighed individually, and preserved in formalin–acetic acid–alcohol. Later, in order to determine the sequence of events during the early stages in growth of a cube, additional cultures were cut with dicer A and placed under the same experimental conditions. These cultures were removed at 2-day intervals over a 12-day period and were preserved in Navashin's solution.

Results

Morphology and histology

Growth of the cultures from small cubes during the course of 9 weeks is illustrated in figures 1–4 and is represented diagrammatically in figure 16, which shows how the cultures changed from cubes into somewhat flattened hemispheres.

When a tissue cube was placed on the nutrient medium, the first divisions of cells occurred by the fourth day (fig. 9). All stages of mitosis were found in the upper portion of the cube to a depth of about seven cells. Further divisions resulted in the proliferation of rows of cells (figs. 5, 10), which became organized into knob-like protuberances (figs. 3, 5, 6).

By the end of the second week the cubes had visibly changed in appearance, the exposed surfaces being covered with small knobs, or bumps, about 1 mm. or less in diameter. As the cultures grew larger, these knobs increased in number.

TABLE 1

Dicer	Distance between blades (mm.)	Range (mg.)	Mean (mg.)	Standard deviation	Coefficient of variability (%)
A...	1.7	4.0– 6.0	5.23	0.521	10.0
B...	2.7	18.0–22.0	19.60	0.732	3.7

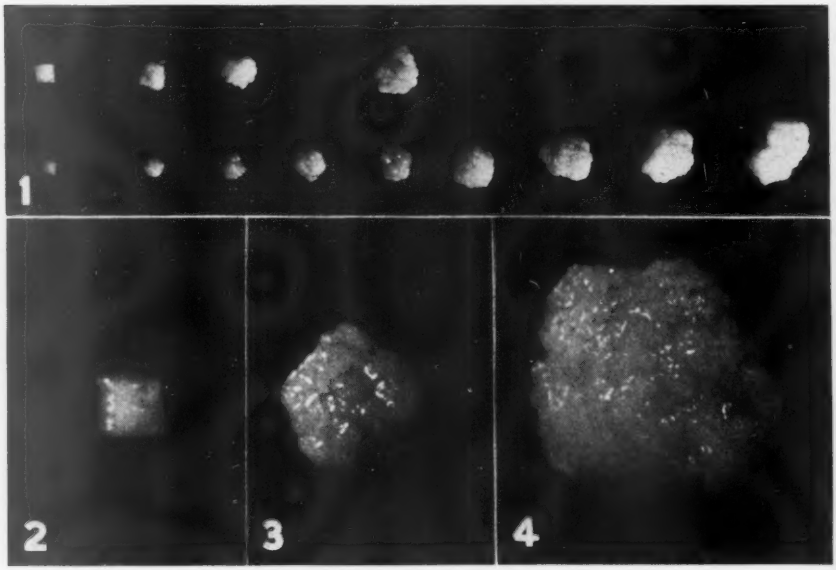
On a 4-week-old culture they were rounded and more or less regularly spaced (fig. 3); later the knobs were crowded together as if in competition for the surface available (fig. 4). As the cultures grew still older, the knobs often became obscured by the presence of dead cells, which gave to the surface a white, granular appearance.

Sections through cultures from successive weeks in the growing period indicated that the knobs increased in number in two ways. One method was by a process of cleavage (fig. 7), which appeared to be the result of failure by a cell, or a row of cells, at the surface to continue dividing. Instead the cell (or cells) enlarged greatly, while other cells at the sides continued to proliferate. The other method was by the formation of new knobs in the subsurface of existing ones (figs. 11, 13).

Growth of a knob was accomplished by divisions of cells at and near the sur-

face (figs. 6, 12). In these divisions new walls were laid down tangentially to the surface. The newly formed cells toward the center of the tissue mass increased in size and, in so doing, pushed the actively dividing cells out along a radius. In this manner a knob continued to grow, its cells forming in orderly rows (figs. 5, 6). Not all divisions within the knob, however, occurred tangentially. As examination of figures 6 and 12 shows, the knobs increased not only in length but in diameter as well. This was effected by cell divisions which occasionally occurred radially (figs. 6, 10, 12) and generally in the center of the knob. New rows of cells were thus formed, which account for the fan-shaped appearance of the knobs in radial section. As the knobs grew, they became appressed laterally, and some of the surface cells in the area of contact were crushed flat (figs. 6, 12). Such crushed cell material stained deep red with safranin. Other cells from adjacent knobs formed such intimate contact that, when a culture was snapped in two mechanically, very often the split occurred through knobs rather than around them.

That proliferation from a cube occurred only from the upper portions suggests that some factor is important in determining where regeneration will occur. A meniscus of liquid nutrient reaching to the top edge formed around the cube soon after it was placed on the agar medium. Thus, a low supply of oxygen may have been effective in limiting growth in the lower portions of the culture. Whether polarity plays a role in this response, however, either partially or even entirely, must await future investigation.



FIGS. 1–4.—Largest cultures. Fig. 1, each weekly series; left to right, 0, 2, 3, 4, 5, 6, 7, 8, and 9 weeks. ×1. Fig. 2, freshly cut cube. Fig. 3, fourth week. Knobs on culture are evenly spaced. Fig. 4, eighth week. Knobs appear crowded. Figs. 2, 3, and 4, ×5.

Examination of cross sections of cul-

tures indicated, despite equal amounts of growth, that not all the sub-surface meristems were in an active state of division at the same time (fig. 6). This raises the question as to whether the knobs grew continuously or in an intermittent and possibly rhythmic fashion. No information is available on this point.

Differences among cultures of same age

The growing cubes, though uniform in initial size, varied considerably in weight at each sampling (table 1). Attempts to detect a relationship between histological aspects and size to account for these variations in amount of growth were not very successful. Certain differences among cultures were noted, however, which might provide clues for an explanation of differences in size and which may form a possible basis for further work.

Differences among cultures at any one time were found in their staining reactions. In some cultures the cell walls in the area approximating the original cube (i.e., in the portion remaining which did not proliferate) were stained deeply red with safranin, although the

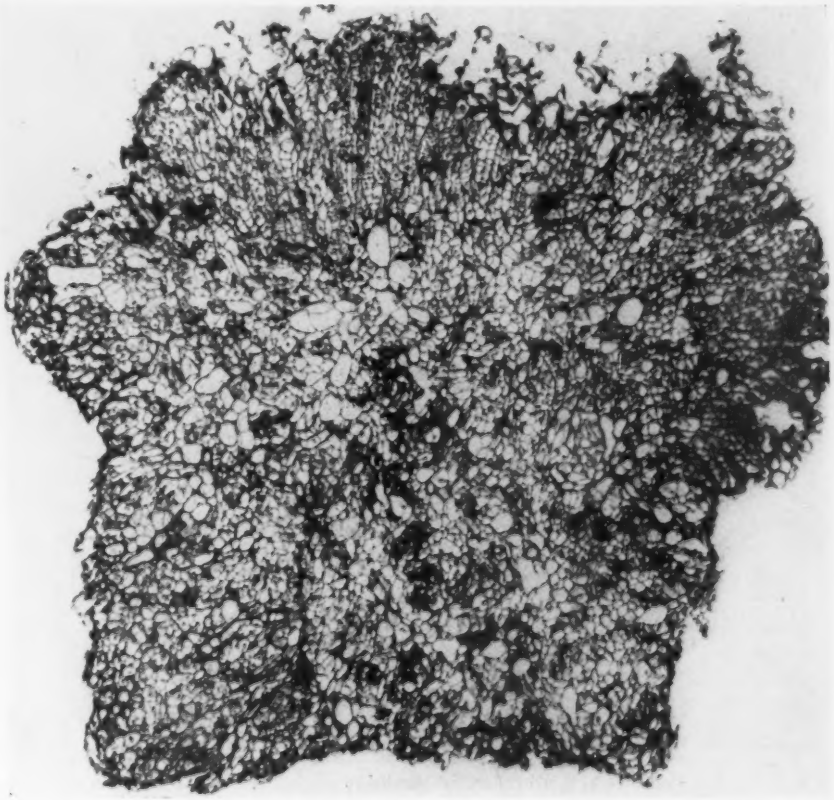
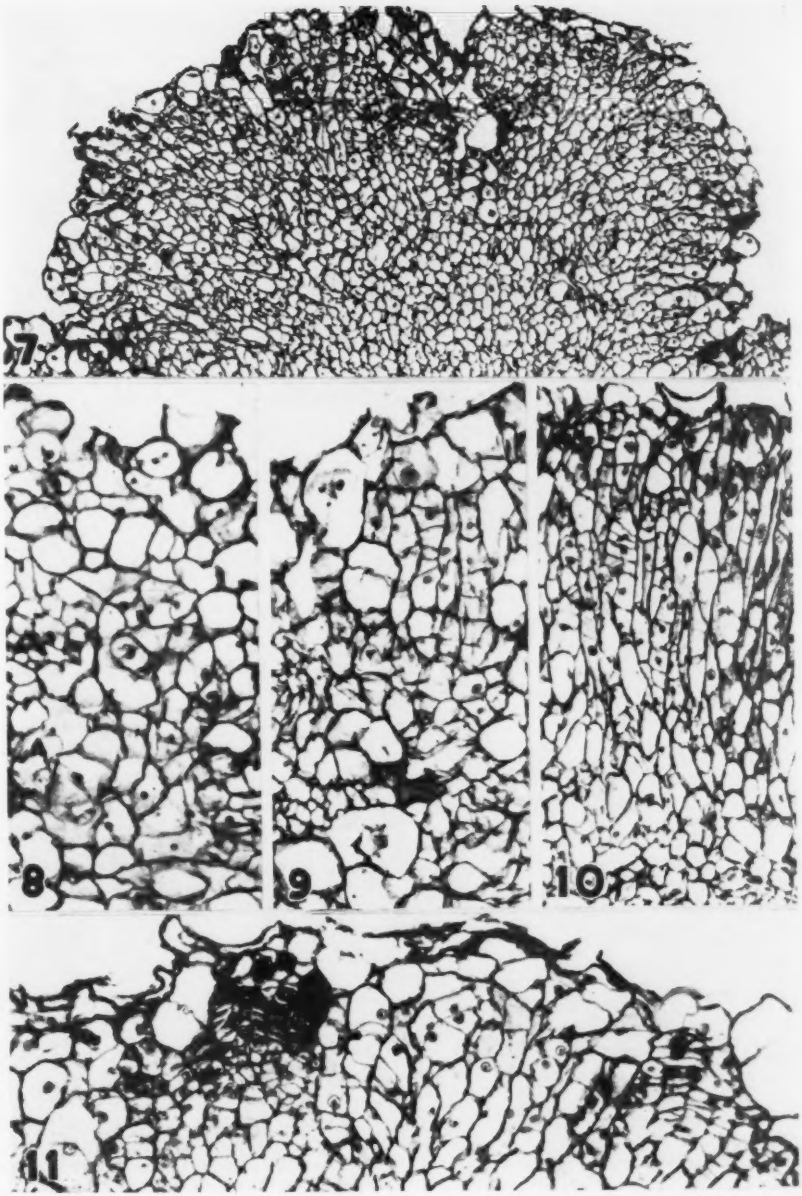


Fig. 5.—Section through 3-week-old culture cut perpendicular to nutrient agar surface, showing that proliferations on culture consist of cells which have been laid down in rows.

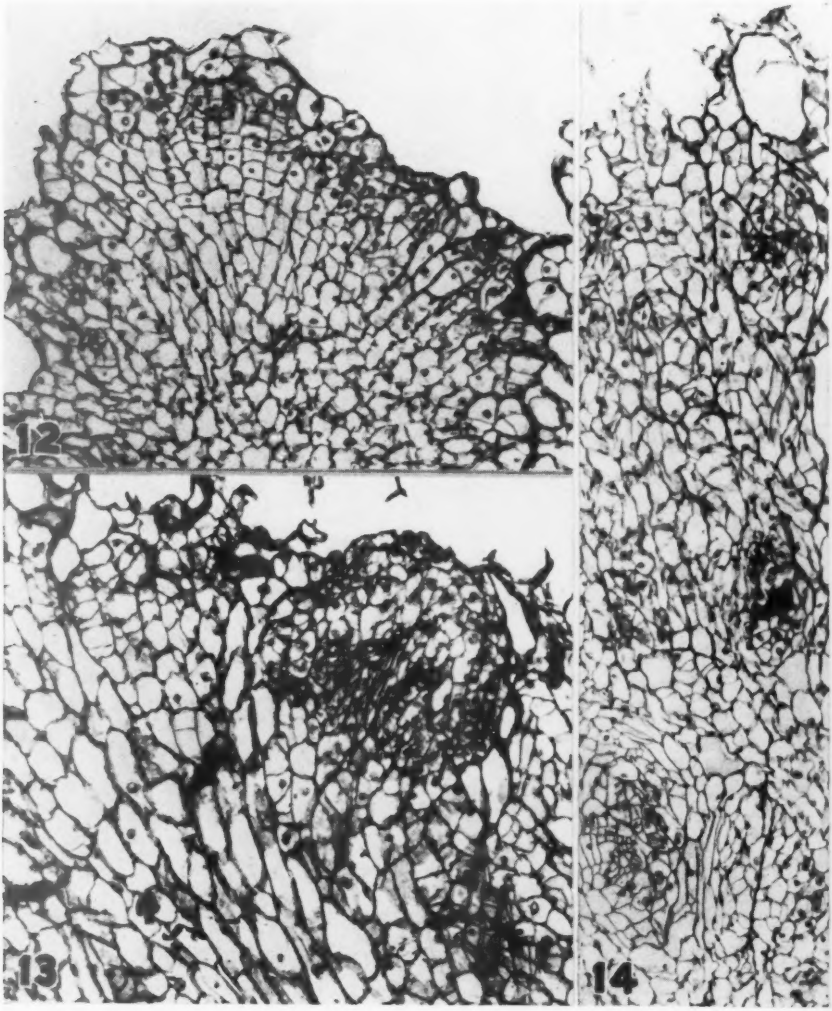
walls of the newly proliferated cells were not. This condition was found in large as well as in small cultures of the same age.

Sections through some of the larger tissue masses in the older age groups showed scattered, apparently dormant, meristematic areas or patches (fig. 14), although the exact way in which they were formed is not clear. Possibly they were cell groups which failed to enlarge after their formation, retaining a meristematic appearance. However, a number of the larger patches seemed to be somewhat active. Mitotic figures found occasionally within these areas indicated that new cells were being formed, and the crushing of a number of cells around these areas indicated that enlargement of some of them was also occurring (fig. 14). Little increase in the weight of a culture, however, can be attributed to the formation of these groups of meristematic cells; if these regions had been

FIG. 6.—Section through 6-week-old culture cut parallel to nutrient agar surface (one-fourth of section shown; lines drawn indicate portion remaining of cube from which culture grew). Orderly growth has resulted from meristematic activity at surface and subsurface of culture. Knob at upper right is in active state of division (see also fig. 12).



FIGS. 7–11.—Fig. 7, knob undergoing cleavage to form two knobs. This process appears to be initiated by failure of one or more of dividing cells in subsurface of growing knob to continue dividing. Instead it enlarges greatly, while neighboring cells continue to divide and grow around it. Figs. 8–10, sections through cultures of different age. Fig. 8, freshly cut cube. Fig. 9, 4 days old; cell divisions tangential to surface have resulted in formation of cells in rows. Fig. 10, 12 days old; continued cell division has produced outgrowth; radial divisions have formed new rows of cells. Fig. 11, new meristematic knob forming in subsurface of older knob.



FIGS. 12–14.—Fig. 12, knob showing cell divisions at and near surface. By continued division and enlargement of cells, structures such as this continue to push out, as shown in figs. 5 and 6. Fig. 13, young knob formed below surface and rupturing tissue of old knob outside it. Fig. 14, section through culture (from ninth week) having a somewhat less orderly histological pattern than culture shown in fig. 6. Organized rows of generally mature parenchyma cells are broken up by patches of meristematic cells formed by continued division of some cells deep in culture. Note crushing of adjacent cells around lower patch.

growing actively enough to contribute appreciably to the increase in weight, breaks and tears, which were not evident, would have been found. Some sections through large, older cultures had a bandlike formation of meristematic cells located along the side of a knob where adjacent knobs were in contact. Of such adjacent knobs the side of only one was found to be meristematic.

The cultures were growing in the dark and were therefore dependent on the medium for carbohydrate. The larger cultures in a given age group were found to contain starch; the smallest ones, however, were found to be free of starch. The areas containing starch were located near the surface in the same general position as the subsurface meristematic cells. In these areas cells with mitotic figures were occasionally found among cells containing large

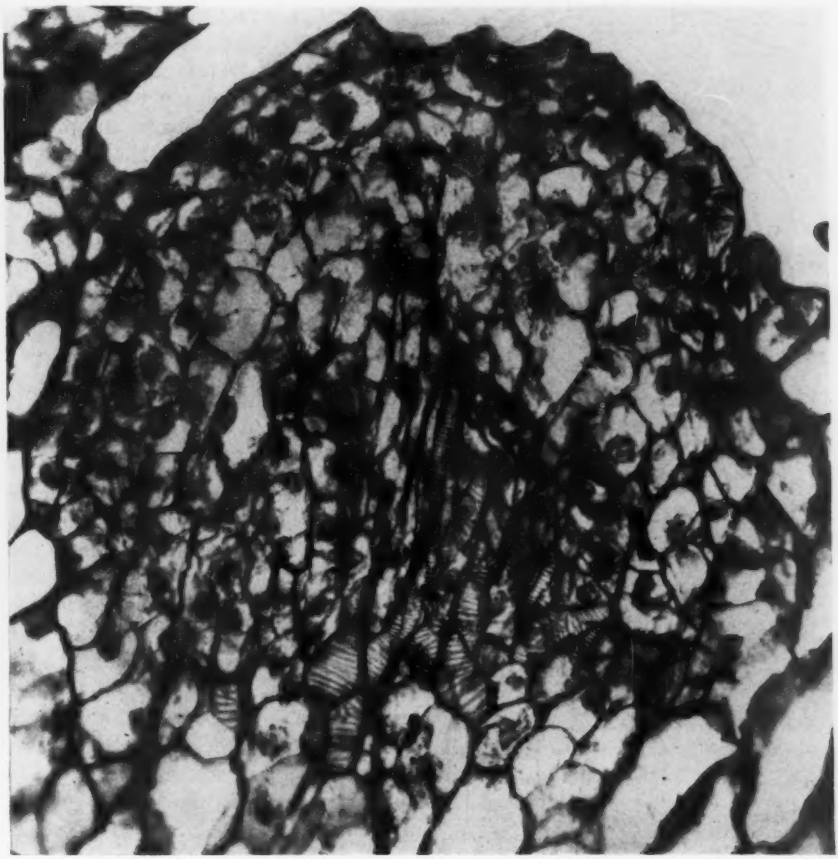


FIG. 15.—Enlargement of young knob in fig. 13, showing "wound tracheids" in center. From serial sections there was no indication of connection between these groups of cells and other similar groups occurring elsewhere in the culture.

starch grains, which is not the normal condition in the apical growing region of angiosperms. It remains to be seen what is the causal connection, if any, between the ability to deposit starch and the greater growth of the large cultures.

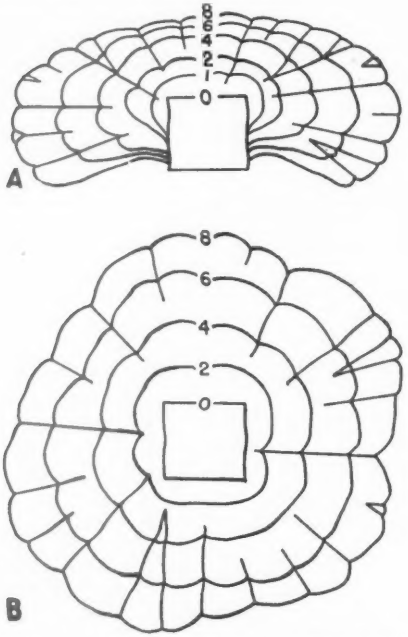


FIG. 16.—Diagrammatic sections through culture illustrating morphological changes during 8 weeks' growth. *A*, section perpendicular to nutrient agar surface; *B*, section parallel to nutrient agar surface. Cubes changed into somewhat flattened hemispheres by growth of knobs in which proliferation of cells occurred in and near surface. Concentric lines represent shape of culture after indicated number of weeks' growth. Radial lines indicate surface of contact between adjacent knobs. Points of radial lines nearest center are points at which knobs have divided. Where two lines radiate from such points, new knobs have been formed.

DIFFERENCES FROM WHITE'S CULTURES

The tobacco cultures first isolated by WHITE grew in a much less organized manner than those here described. A section through an 8-week-old culture illustrated by WHITE (22) shows a relatively uniform central area surrounded by loosely organized groupings of meristematic knobs generally separated from one another by groups or rows of much larger cells. Sections through our cultures show, however, much more evidence of growth in an orderly fashion, whereas growth in WHITE'S original cultures seems to be more randomized. The reasons for these histological differences are not clear. The fact that WHITE'S cultures grew in the light rather than the dark may, however, be a factor.

Another difference between the two sets of cultures concerned the presence of differentiated cells of the wound tracheid type. Whereas in WHITE'S cultures these were present as "isolated" cells, here serial sections indicated that at least for short distances many of these cells were connected together and similarly oriented. Figure 15 indicates that the formation of these cells may occur very early in the development of a newly formed meristematic knob.

WEIGHTS OF CULTURES

The weights of the cultures increased with time, but the increase of the largest culture from each weekly sample, in both the A and the B groups, was much greater than the average increase for each group respectively (table 3). In group A after 9 weeks there had been an 18-fold increase in mean weight, while the largest cultures showed a 34-fold increase; in group B after 5 weeks there had been a 4.8-fold increase in the mean, and a 6.1-fold increase in the largest cultures. This variance also increases with time as the cultures grow. Only three cubes in group A and one cube in group B failed to grow at all.

When the weights of the largest cul-

tures from each weekly group sample were plotted, it was discovered that they fell in regular logarithmic relationship to one another. This indicates not only that growth was proportionately progressive but that the largest culture in each weekly sample of twenty cultures had the *maximum* growth rate in its group; it also had the *same* growth rate as the largest culture from each successive weekly sample. Each point of each growth curve in figure 17 represents, therefore, the growth attained by a single culture of maximum growth rate under the conditions of the experiment. The largest cultures in successive weeks in the A and B series are shown in figure 1. The growth curve of the A cultures shows an anomalous weight at 2 weeks, which at present cannot be accounted for except by assuming that the particular culture up to that time had a growth rate the same as the largest cultures in the other weeks and that its initial weight (which was not measured) was approximately 20% greater than the largest measured initial weight at time of inoculation.

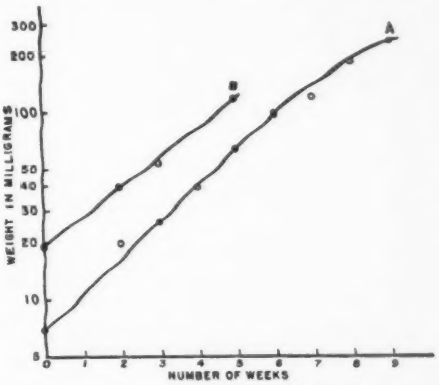


FIG. 17.—Log of wet weight in relation to time of largest cultures in A and B series.

Discussion

TISSUE GROWTH AND COMPOUND-INTEREST LAW

BLACKMAN (1) has interpreted the addition of new material by a plant in terms of the compound-interest law. The equation which BLACKMAN found applicable for the growth of an annual plant is

$$W_1 = W_0 e^{rt},$$

where W_1 is the final weight, W_0 is the initial weight, e is the base of natural logarithms, r is the rate of interest, and t is the time. The rate of interest is expressed as percentage increase per day. It is considered by FISHER (3) not only to be a valuable index for comparing the efficiency of different plants over comparable times and phases of development but as the correct measure for the mean value of the relative growth rate over any period, long or short. For a constant growth rate during a time interval this would be a way of describing numerically the relative rate of growth; for a fluctuating or changing rate of growth during the time interval, such as the alternating growth rate in day and night or a change in average daily growth rate during a growing period, this would represent an average relative rate of growth for the period. BLACKMAN found that rates of interest for a number of plants, calculated from the data of other workers, ranged from 10.6 to 20.5% per day.

The use of BLACKMAN's formula appears to be particularly applicable for describing the growth of the writer's tissue cultures, since under the easily maintained uniform conditions of the experiment the weights of the largest cultures appear for the first 5 or 6 weeks to indicate constant exponential growth.

Applying the formula, which is more

convenient in the form $\log_e (W_1/W_0) = rt$, r may be calculated as shown in table 2.

The relative growth rates of 6.3% and 5.15% per day represent the maximum rates of growth of the cultures of different initial size under the conditions of the experiment. It should be noted here that the tissues were not subcultured weekly but were growing continuously in one place on the nutritive medium.

Thus far little attention has been given to the quantitative interpretation of the growth rates of plant tissue cultures.

TABLE 2

Group	No. of days (t)	Relative increase	r
A.....	42	14.2	0.063
B.....	35	6.1	0.0515

However, some data showing weight increase of tissues in a given time are available. NOBECOURT (14) reported that a 1-mg. fragment of carrot root tissue increased to 185 mg. in 5 months, with a transfer to fresh nutrient after the first 3 months. The rate of increase calculated from BLACKMAN's formula was 3.48% per day. GAUTHERET (5) measured the increase in weight during the eighth monthly passage of eight pieces of carrot root tissue. These varied in size at the beginning of the culture period from 50 to 180 mg. Average rates of increase for 58 days ranged from 2.8 to 4.9% per day. Data on three tissues from the ninth passage were also given. In 18 days the most rapidly growing culture grew from 125 mg. to 445 mg.—an increase of 7.05% per day. Later he reported that a 0.5-mg. fragment of carrot tissue increased to 15 gm. in a period of 10 months—an increase of 30,000 times. This represents an increase of 3.4% per day. Since nothing is known in these cases concerning the growth curves during the culture period, it is assumed that these were the average growth rates and that the maximum rates of growth may have been greater. However, the calculated figures represent the maximum rates of growth if the rates of increase were constant.

In his paper describing the first successful culture of *Nicotiana* callus, WHITE (22) reported an average 3-fold increase per passage of 7 days. No figures were cited. Growth on this basis is a rate of increase of 15% per day, a figure comparable with that for the relative growth of an intact plant. Additional information concerning growth rates in plant tissue cultures must await further investigation.

VARIATION WITHIN GROUPS IN GROWTH RATES OF CULTURES

Table 3 shows that weights of cultures in groups A and B increased progressively in variability as the experiment proceeded. Initially all the cultures within each group were approximately the same size and shape. Had they been growing at the same rate, one would, at the end of a growing period, expect the variability of the cultures—expressed in standard deviation—to increase only in proportion to the increase in weight. The coefficient of variability would therefore have remained constant. This, however, was not the case. As the cultures increased in age, their variability increased considerably in both groups A and B. This effect was so pronounced that by the end of the eighth week the weight ratio of the largest to the smallest culture in group A was 10:1.

This increase in variability may be accounted for by either one of the following hypotheses: (*a*) the cultures were all growing at different rates or (*b*) they were all growing at the same rates but

different cultures stopped growing at different times after the beginning of the experiment.

Work on this phase of the problem is at present in a preliminary stage. Mention may be made, however, of an experiment in which tobacco callus cultures having an initial weight of 9 mg. or less were grown and weighed individually at weekly intervals during the growing period. The data collected seem to indicate that (*a*) is the correct hypothesis. Of fourteen pieces cultured for 11 weeks, none had stopped growing during that time. All cultures had increased progressively in weight. At the end of 11 weeks the ratio of the largest to the smallest culture in the group was as 2088:210 mg., or 10:1. At the beginning of the experiment these cultures had weighed 8.8 mg. and 8.4 mg., respectively. Of these, one culture had increased 238 times, the other only 25 times. It seems, therefore, that small cultures of this tobacco callus possess inherent differences which are independent of initial size.

On the basis of these observations the differences in growth rates of initially small cultures must be due to inherent differences in the tissue cubes at the time that they were cut. To what extent these differences between tissue cubes are associated with their original sites or orientation in the tissue mass when cut, or with the growth rate of the culture from which they were subcultured, or to what extent the age of a culture is a factor in regulating subsequent growth rates, are questions yet to be investigated.

Differences in growth rate in relation to initial size of explant

Examination of figure 17 indicates that the growth curves do not have the same slope. Cultures of larger initial size (B) have a smaller relative growth rate. The difference between the two slopes is 1.15% ($P = < 0.005$ for $N = 6$). Although this percentage may seem small, such a difference can have a profound effect on the final weight. As Blackman (1) pointed out, a difference of 1% in relative rates of growth will cause a 2-fold difference in final weight at the end of 69 days, when the rates of growth have remained constant. This suggests that cultures of still smaller initial weight and possessing the same

TABLE 3

Growth of uniformly cut cultures of tobacco callus

No. of weeks' growth	No. of cultures removed	Weight of largest culture (mg.)	Mean weight (mg.)	Standard deviation	Coefficient of variability (%)
		A cultures			
0	20	6.8	5.8	0.61	10.5
2	20	19.9	10.6	3.28	31.0
3	20	26.0	17.6	4.74	26.8
4	20	39.0	19.8	6.26	31.7
5	20	63.8	36.6	16.80	46.0
6	20	96.7	46.4	22.30	48.0
7	16	119.6	72.0	27.60	37.8
8	16	184.0	93.0	37.50	40.3
9	16	231.4	105.0	57.20	54.5
		34×*	18×*		5×*
		B cultures			
0	20	19.1	17.2	0.75	4.6
2	19	39.7	32.7	3.38	9.4
3	16	53.8	43.4	6.27	14.5
5	16	116.0	74.4	22.30	30.0
		6.1×†	4.8×†		6.5×†

* Increase in 9 weeks.

† Increase in 5 weeks.

physiological potentialities would show an even greater relative growth rate.

GAUTHERET (6) has presented evidence which supports this point of view. Using cultures of carrot root tissue, he found that the ratio of the final to the initial weight of a culture varied inversely as the initial weight. From data in his earlier paper (5) on carrot tissues grown for 58 days, it can be shown by arrangement of his data in order of increasing initial weight—50, 50, 110, 115, 150, 155, 160, and 180 mg., with final weights of 585, 735, 1180, 815, 1265, 1270, and 915 mg., respectively—that the relative rates of growth fall into a generally descending series as follows: 4.95, 4.63, 4.10, 3.36, 3.74, 3.62, 3.57, and 2.81%, respectively.

The concept that cultures of smaller initial weight tend to grow relatively more rapidly is further supported by the work of BUCHSBAUM (2) in studies with embryonic cells of the chick. He showed that smaller explants had greater relative increase in 48 hours than larger explants. He attributed this slower growth in larger explants to the inhibiting effect on peripheral cells of the more unfavorably located interior cells, owing to slow diffusion of nutrients and metabolic products.

That these factors operate in plant tissue cultures as well is doubtless true. However, they do not appear to be the complete answer, for, as figure 17 indicates, cultures of the same weight from each of the curves maintained different growth rates for well over 3 weeks. Yet there is every reason to believe that the cultures of maximum growth rate in each group were initially of the same physiological constitution and differed only in size. This difference in growth rate may perhaps be accounted for by the difference in shapes of cultures of similar weight in the respective groups; at 20 mg., for example, culture B was approaching a hemisphere in shape, while culture A was still a cube.

Summary

1. The growth in darkness of tobacco stem callus tissue on nutrient agar medium was investigated. Growth was studied during a period of 9 weeks in cultures taken from stock subcultures which were 6 weeks old. The experimental cultures were prepared by cutting the stock cultures into uniform cubes of two different sizes by a technique which is described.

2. Increase in size of the cultures resulted from the proliferation of small knoblike protuberances in which division and enlargement of cells was occurring very close to the surface. Knobs increased in number in two ways: (*a*) by cleavage and (*b*) by the formation of new growing centers in the subsurface of older knobs. An account of the histology of the growing cultures is presented.

3. Though uniform in initial size and under constant cultural conditions, the cultures within each series grew at different relative rates. Thus there were differences inherent in the initial culture pieces which determined the subsequent rates of growth of the cultures.

4. Over and above the large amount of variation in growth rate of cultures started from pieces of the same size, there were differences in growth rate attributable to the initial size of the explant. The smaller the explant, the greater was the relative growth rate.

5. For each series growth curves were plotted by reference to the largest culture at any time. In these terms the cultures increased exponentially with time for 5–6 weeks. Growth rates are

described in terms of BLACKMAN's formula.

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DEPARTMENT OF BOTANY
UNIVERSITY OF CHICAGO

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DEVELOPMENT OF OVULE AND MEGAGAMETOPHYTE IN POMEGRANATE

J. R. KING[1]

Introduction

There appeared in 1935 a publication entitled "Development and vascular anatomy of the flower of *Punica granatum* L." by CHIH-LUNG TUNG (**8**). Just prior to this, there was completed in the Division of Pomology at Davis a similar study by W. B. HEWITT (unpublished). Since these two studies substantiate each other almost completely, it has been considered unwise to publish the work of HEWITT. TUNG reported, however, that the outer, or upper, whorl of carpels arises immediately after the initiation of the petal primordia and numbers from four to eight; HEWITT found that this whorl arises at about the same time as the petal primordia and numbers four to twelve. Likewise, TUNG mentioned that the innermost, or third, whorl of carpels consists of only one carpel or is, in most cases, absent. HEWITT, on the other hand, found more than one carpel in the innermost whorl, but seldom were there more than two.

Since neither of these workers investigated the development of the megagametophyte in pomegranate, such a study was made here before the war. This paper is a report of that work and serves to round out more completely the story of floral development in the pomegranate.

Investigation

The material used was collected from trees of the Wonderful variety growing near the University Farm at Davis. The ovaries were fixed in both Navashin's fluid and formalin–acetic acid–alcohol, dehydrated and cleared by the alcohol-xylol method, and imbedded in paraffin. Sections were cut 12–18 μ thick, depending upon the size of the material, and were stained with either iron alum–hematoxylin or safranin and fast green.

The ovules arise opposite vascular branches and are first indicated by the division of one to several cells in the hypodermal layer. These small groups of ovule initials are distributed indeterminately over the entire placental region of the carpel. Succeeding divisions cause a protrusion into the locule. These occurrences are substantiated by TUNG (**8**) and in the unpublished work on pomegranate by HEWITT.

The young ovule is projected by the short funiculus beyond the placental surface. Greater growth on one side, however, causes the ovule gradually to bend, so that at maturity it is in the anatropous position. The bending may be either to the left or to the right. Usually, however, the ovules in one half of the locule are turned in the opposite direction from those in the other half. Ovules near the center of the carpel are older than those near the outer edges.

The origin of the inner of the two integuments is first evident as a division of an epidermal cell of the ovule followed by division of adjacent epidermal cells at the same level. This ring of meristematic cells divides toward the tip of the ovule. The ovule, at the time of initiation of the inner integument, has curved until the tip is almost parallel with the placenta. The outer integument is initiated

[1] Assistant pomologist, University of California, Davis, Calif.

in the same manner and at approximately the same time as the inner, but originates four or five cells below the latter (fig. 1). As the ovule continues to develop, the greater growth of the outer integument causes it to surpass the inner.

The archesporial cell arises usually as the fourth cell beneath the epidermis soon after the integuments begin growth. It is distinguished by its large size, dense cytoplasm, and large centrally placed nucleus. The archesporial cell becomes the megaspore mother cell directly, and no parietal tissue is formed. Such a change is accompanied by increase in size and by elongation (fig. 2). Its frequent later position deeper in the nucellar tissue is brought about by division of overlying nucellar cells rather than by the formation of parietal cells.

The meiotic division of the megaspore mother cell in the formation of the two daughter cells (fig. 3) is accompanied by a slight breakdown of surrounding nucellar tissue. The second division results in a linear group of four megaspores.

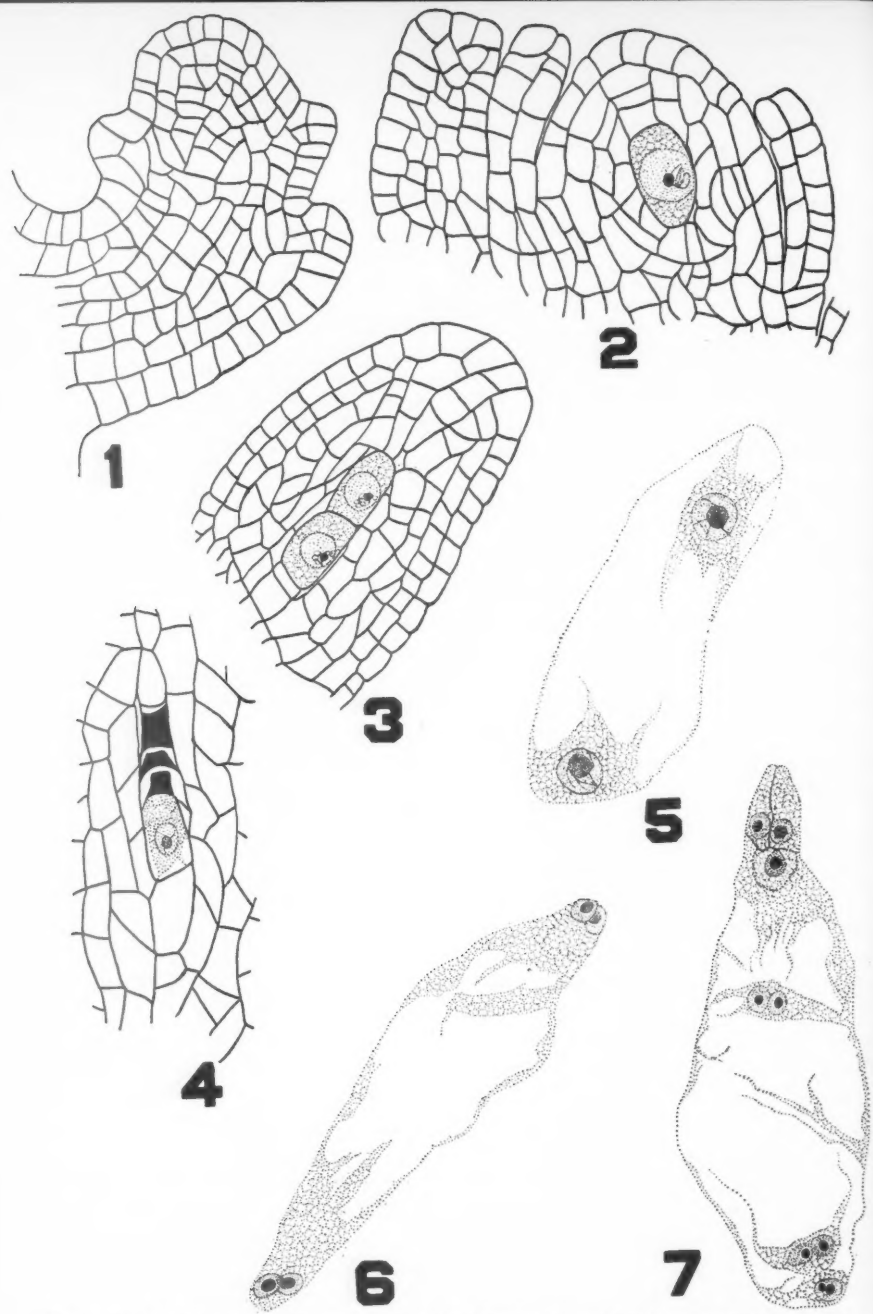
Only the chalazal megaspore takes part in the formation of the megagametophyte. The upper three megaspores are nonfunctional and gradually degenerate into dark staining, structureless masses (fig. 4). They later disappear, and their places are taken by the developing megagametophyte. Although the early changes and nuclear division of the one-nucleate megagametophyte could not be followed closely, the two-nucleate condition presents a much enlarged megagametophyte both in length and in width. The two free nuclei, one in each end of the developing megagametophyte, are separated by a large vacuole (fig. 5). The second nuclear division, which results in the four-nucleate condition, is accompanied by further increase in size of the megagametophyte and of the vacuole. The four free nuclei are usually present as a single pair in each end of the megagametophyte (fig. 6). Occasionally, however, they assume positions about equally distant from one another.

As the ovule matures, the outer integument exceeds the inner in length and width and finally envelops the ovule, except at the micropyle. The outer integument is usually from four to eight cells in width midway between the micropyle and chalaza but widens toward the micropylar end. It may in later stages separate from the nucellus and inner integument. The latter two, however, remain appressed except at the micropyle. The inner integument remains two cells wide for most of its length but broadens near the micropylar end.

The nucellus gradually disintegrates as the megagametophyte enlarges; however, when the latter is mature, nucellar tissue is still present in scattered areas.

The vascular trace, which enters the funiculus and extends through the raphe, splits into three or four branches as it nears the chalaza. Each branch diverges usually at about the same level. The main trace continues through the raphe and into the chalaza, where it may branch again.

Nuclear division in the four-nucleate megagametophyte results in a typical eight-nucleate condition, with a group of four nuclei in each end separated by a large vacuole. Early migration of one nucleus from both the micropylar and the chalazal regions toward the center of the megagametophyte is followed soon by cell-plate formation which results in a typical seven-celled, eight-nucleate megagametophyte, although the antipodal cells degenerate very early (fig. 7). The shape of the mature megagametophyte varies considerably, especially in



FIGS. 1–7.—Camera lucida drawings. Stages in development of ovule and megagametophyte. Fig. 1, developing ovule showing origin of inner and outer integuments. ×575. Fig. 2, megaspore mother cell in young ovule develops directly from archesporial cell without formation of parietal tissue. ×700. Fig. 3, two daughter cells following reduction division of megaspore mother cell. ×700. Fig. 4, three degenerating megaspores and one developing megaspore in linear arrangement. These four megaspores result from second meiotic division of spore mother cell. Developing chalazal megaspore becomes young megagametophyte. ×700. Fig. 5, two-nucleate megagametophyte. ×575. Fig. 6, four-nucleate megagametophyte. ×575. Fig. 7, seven-celled, eight-nucleate megagametophyte, consisting of two synergids, one egg, two polar nuclei, and three antipodals. Antipodal cells shown have already begun to degenerate. ×575.

width. More often it is rather broad just below the center, then tapers toward both the micropylar and the chalazal ends.

The two synergids of the egg apparatus are elongated. The nucleus of each lies in the lower portion and on the same focal plane. A conspicuous vacuole, either above or below the nucleus, was very seldom evident; however, a less dense cytoplasmic area usually does exist above the nucleus.

The egg is frequently situated slightly below and midway between the two synergids; however, this position varies. In some instances it may be found beneath either the right or the left synergid. In *Carya glabra* the synergids have occasionally been found to the left of the egg when the section is cut perpendicular to the plane of the middle septum (**4**). The egg is usually not distinct from the synergids in having a larger nucleus or more dense cytoplasm. A similar condition exists in *Saxifraga* (**1**). The egg nucleus in pomegranate varies in size. Sometimes it is larger and sometimes it is smaller than those of the synergids or polar nuclei.

The two nuclei which are to become the polar nuclei migrate early from each end of the megagametophyte and lie close to one another, usually near the center of the megagametophyte They fuse before fertilization. Frequently they are found in contact or fused near the side of the megagametophyte or in the micropylar end near the egg apparatus, as in *Lycopersicum* (**2**). The large nucleoli remain distinct after the cells fuse.

The true nature of the mature megagametophyte in pomegranate is rather difficult to determine because of the early beginning of degeneration of the antipodal cells. In all the mature megagametophytes observed, at least a slight degree of degeneration was apparent. The position and appearance of the antipodals vary considerably. One of the three is usually nearer the chalaza, and the group forms a triangle. The triangle may be inverted in shape, with two antipodals nearer the chalaza than the third. Occasionally the three cells are on nearly the same level. In any event, degeneration begins first in the lowest of the three cells and is soon followed by degeneration in the upper ones. The disappearance of the cell wall is an early indication of antipodal cell degeneration.

Discussion

SCHNARF (**6**) in 1931 stated that the embryological behavior of the Punicaceae was entirely unknown, and a survey of the literature on megagametophyte development has revealed no reference to such studies since that time. It is not possible, therefore, now to make comparisons with other members of the family. The characteristics involved in the formation of the megagametophyte of the pomegranate are those of the "normal" type, which is characterized chiefly by the fact that there occur five successive nuclear division stages in the origin of the egg cell from the megaspore mother cell and also by the fact that only one megaspore is involved in the formation of the megagametophyte. Considerable variation exists in the various phases of this type of megagametophyte development—for example, in the formation of the megagametophyte, in the divisions of the megaspore nucleus, and in the organization of the mature megagametophyte.

Discussions of the normal type of megagametophyte development and its many variations have been presented and have been ably reviewed by others (**3**, **5**, **7**).

Summary

The ovule of pomegranate is anatropous. The formation of the megagametophyte is of the "normal" type, which is characterized by the fact that there occur five successive nuclear division stages in the origin of the egg cell from the megaspore mother cell and also by the fact that only one megaspore is involved in the formation of the eight-nucleate megagametophyte.

DIVISION OF POMOLOGY
UNIVERSITY OF CALIFORNIA
DAVIS, CALIFORNIA

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ETHER-SOLUBLE ORGANIC ACIDS AND BUFFER PROPERTIES OF CITRUS PEELS[1]

WALTON B. SINCLAIR AND DESIRE M. ENY

Introduction

The fact that the pH of the peel of citrus fruits (pH 5.0, or greater) is much higher than that of the corresponding juices of the pulp (**10, 11, 12**) indicates that the buffer properties of the two systems are quite different. Owing to the high pH, the organic acids present in the peel exist chiefly in the salt form and not in the free state. Furthermore, the high pH values of the peel strongly indicate that the buffer system is composed of salts of inorganic acids and not of organic acids and their salts.

Previous investigations (**13, 14**) have shown that the buffer system of citrus juices is composed of organic acids and their salts and that these juices are similar to pure citric acid solutions containing equivalent amounts of free acid and salt of a strong base with a common anion. No positive statement can be made with respect to the buffer system of the peel. So far as the authors know, no thorough study of the buffer systems of citrus peels has been made. Such data are essential, however, for the understanding of certain physiological relations between peel and pulp and for the understanding of the metabolic reactions of the peel under storage conditions.

The present studies were carried out to determine what constituents of the peel of oranges, grapefruit, and lemons

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are responsible for the buffer capacities. The experiments involved the determination of the ether-soluble organic acids both in the extract of the fresh peel and in the dried peel. The organic acids (citric, malic, and oxalic) of the peel extract were determined both on a lead acetate precipitate and on an ether extract. Titration curves for the peel extract of oranges, grapefruit, and lemons are presented so that a comparison can be made between the buffer properties and the organic acids that occur in the peel. From these data, the relation of pH to the free and the combined acids can be determined.

Material and methods

Preparation of samples

The samples (fifty to one hundred fruits each) of mature navel and Valencia oranges, grapefruit, and lemons were taken from plots of trees located in experimental orchards at the University of California Citrus Experiment Station and in commercial groves in the vicinity of Riverside, California. The peel was carefully removed from the fruit to avoid contamination with the juice. As the juice of citrus fruits is high in organic acids, contamination of the peel with a small amount of juice from the vesicles of the pulp would increase excessively the total acid content of peel samples.

The peel extract was prepared by cutting the fresh peel into small pieces, placing them in glass jars with covers, and then freezing for 48–60 hours at −25° C. After thawing, the peel was quickly ground through a galvanized meat-chopper. The juice was extracted from this ground mass by means of a hydraulic press, at a pressure of 20,000 lb./sq. in. The liquid obtained will be referred to in the remainder of this paper as the peel "sap."

The dried-peel samples were obtained by taking aliquot portions of the ground peel, and of other peel samples that were not frozen, and drying them in a mechanically ventilated oven at 65° C. The dried peel was prepared for the analyses by grinding to a fine powder in a Wiley mill.

For moisture determinations, known amounts of fresh peel were weighed in small aluminum containers.

Chemical methods

Preliminary experiments early showed that the individual organic acids could not be determined accurately on the peel sap in the presence of the large concentration of pectin but had to be determined on a pectin-free extract. To precipitate the pectins, sufficient ethyl alcohol was added to a known volume of sap to produce a final concentration of 85% alcohol, and the precipitate was separated from the alcoholic solution by either filtering or centrifuging.

Ether liquid extraction.—In the use of this alcoholic solution for the isolation of the organic acids with ether by the liquid extraction method, the alcohol was completely driven off on a steam bath without allowing the solution to become dry. This was done by evaporating to a very low volume, adding distilled water frequently, and re-evaporating until the solution was free of alcohol. The resulting solution, free of both pectin and alcohol, was diluted with distilled water to the original volume of the sap. It was then subjected to a continuous ethyl ether liquid extraction in a Bailey-Walker apparatus modified to include a small funnel and a glass thimble to hold the liquid, instead of the usual syphon cup. As the funnel rested on the bottom of the thimble, the condensed ether passed through the column

of the liquid sample, and complete extraction occurred in between 24 and 30 hours. A small amount of water was then added to the ether extract, and all the ether was carefully evaporated off, leaving the organic acids in the aqueous phase. This liquid was filtered and subsequently diluted to a known volume. Aliquots were used for determining the ether-soluble organic acids in the peel sap.

LEAD ACETATE PRECIPITATION.—In supplementary comparative determinations, the organic acids were precipitated by lead acetate from the pectin-free alcoholic solution (85%) referred to earlier, according to the procedure of HARTMANN and HILLIG (3). The precipitate was washed with alcohol, suspended in water, and freed of lead by passing H_2S through the solution. The lead sulfide was filtered off and washed with water. The filtrate and washings were combined and diluted to a known volume. Aliquots of this solution were used for determining the organic acids in the peel sap which had been precipitated by lead acetate.

EXTRACTION OF DRIED PEEL.—Organic acids were isolated from dried peel by the method of PUCHER *et al.* (6). A known weight of ground dried peel was acidified to pH 1 with sulfuric acid (4*N*), mixed thoroughly with asbestos, and subsequently extracted with absolute ether (H_2O_2-free) for 30 hours. Water was added to the extract, and the ether was carefully evaporated from the solution, leaving the organic acids in the aqueous phase. This solution was filtered into a volumetric flask and diluted to final volume. Aliquots were used for the analysis of the organic acids.

DETERMINATION OF ORGANIC ACIDS.—With a Beckman glass-electrode pH meter, the total organic acids were determined by titrating the extracts between the limits of pH 7.8 and 2.6, according to the method of VAN SLYKE and PALMER (15). Since oxalic acid reacted as a monobasic acid under these conditions, a correction was made by determining independently the amount of oxalic acid in the samples. Suitable aliquots, depending upon the oxalic acid content, were measured into 100-ml. beakers and acidified to Congo red with 0.5*N* HCl. The material that flocculated on standing was filtered off on a fine-sintered crucible and washed with water. The precipitation and titration of the oxalate from this clear liquid were carried out according to the procedure of PUCHER *et al.* (6).

Quantitative determination of citric acid, free and combined, was made by the pentabromacetone method of PUCHER *et al.* (7). The sample was heated with H_2SO_4 to convert the combined citrates to free citric acid, and the citric acid was oxidized to pentabromacetone by $KMnO_4$ in the presence of KBr. After extraction of the pentabromacetone with petroleum ether, the bromide ion was liberated with Na_2S and was subsequently titrated with standard $AgNO_3$. The citric acid in the original sample was calculated from the titration.

Malic acid was also determined by the pentabromacetone method of PUCHER *et al.* (7). The principle of the method involves the oxidation of malic acid with $KMnO_4$, in the presence of KBr, to a bromine compound volatile with steam. This compound reacts with 2,4-dinitrophenylhydrazine to give a water-insoluble product which is soluble in pyridine. The pyridine solution of this substance, when correctly diluted with water and made alkaline with NaOH, develops a blue color proportional to the amount of malic acid present.

Titration curves of both normal and pectin-free peel sap were determined at 23° C. with a Beckman glass-electrode pH meter.

Results

Organic acids of peel sap, determined by the ether liquid extraction method

The data in table 1 show the amounts of the various organic acids obtained from peel sap by continuous extraction with absolute ether. The relatively high pH values of the sap indicate that 75–80% of the organic acids are in the salt form. As the extraction was performed at pH 1, the ether extract of the sap included, in the free form, the total organic acid radicals. The values in column 5 of table 1 show the milliequivalents per milliliter (me./ml.) of organic acids, or the total organic acid, extracted from the sap with ether and subsequently determined by titration between pH 7.8–2.6, with corrections for oxalic acid and other variables. The amounts of organic acids in the peel saps of citrus fruits are very low in comparison with those in the juices of the pulp (11, 12).

The total polybasic acids extracted from the peel saps with ether are represented chiefly by citric and malic acids (table 1). A trace of oxalic acid was present. The relatively high pH values of the saps indicated that the oxalic acid existed chiefly as an insoluble salt and did not occur in appreciable concentrations in the centrifuged saps. The sum of the milliequivalents of citric and malic acids agreed satisfactorily with the total organic acids (milliequivalents) titrated between pH 7.8 and 2.6. This is good evidence that if other nonvolatile organic acids were extracted with ether, they were present in the sap in only microquantities. In the peel sap the malic acid concentration is much higher than that of citric acid; in citrus juices, the reverse order prevails.

Organic acids of peel sap precipitated with lead acetate

The completeness with which lead acetate precipitated the organic acids and acid salts is made evident by the close agreement of the sum of the milliequivalents of citric and malic acids with the total-acid values (titrated between pH 7.8 and 2.6) of the ether extract (table 1). The citric and malic acid values of the ether extract agree substantially with similar values of the lead-acetate precipitate. The close agreement of the values determined by these two entirely different methods indicates, to an accuracy of 5%, the actual organic acid content of the peel sap. The conclusion can also be drawn that the liquid extraction method is satisfactory for the isolation of organic acids from citrus-peel saps.

Ether-soluble organic acids of dried citrus peels

The data in table 2 report the amounts of organic acids (mg./gm. fresh weight) in the peels of navel and Valencia oranges, lemon, and grapefruit. Although data are reported on a fresh-weight basis, actual determinations were made on the ether extract of dried peel. In these determinations the organic acid content was greatest in navel orange peel and least in grapefruit. The concentrations of total acids in the peels of Valencia orange and lemon fell between these two extremes. Although the relatively larger amounts of organic acids in navel orange peel cannot be explained, it should be mentioned that these peel samples were more mature than the others. The smaller amount of organic

TABLE 1

ORGANIC ACIDS OF PEEL SAP OF CITRUS FRUITS, DETERMINED BY LIQUID EXTRACTION AND LEAD ACETATE PRECIPITATION METHODS

Sample no. and variety	Sampling date (1946)	pH of sap	Free acid of pectin-free sap	Ether-soluble organic acids						Acid constituents precipitated by lead acetate				
				Total organic acids (pH 7.8–2.6)	Citric acid		Malic acid		Total citric and malic acids	Citric acid		Malic acid		Total citric and malic acids
			me./ml.	me./ml.	mg./ml.	me./ml.	mg./ml.	me./ml.	me./ml.	mg./ml.	me./ml.	mg./ml.	me./ml.	me./ml.
1. Navel orange	Feb. 26	5.00	0.022	0.052	1.22	0.019	2.35	0.035	0.054	1.54	0.024	2.28	0.034	0.058
2. Navel orange	Mar. 11	5.05	.024	.055	0.58	.009	2.82	.042	.051	0.38	.006	3.08	.046	.052
3. Navel orange	Apr. 2	5.40	.015	.029	0.38	.006	1.41	.021	.027	0.32	.005	1.34	.020	.025
4. Lemon	Apr. 17	5.38	.015	.020	0.32	.005	1.01	.015	.020	0.32	.005	1.01	.015	.020
5. Lemon	Apr. 27	5.48	.014	.020	0.19	.003	0.94	.014	.017	0.26	.004	1.01	.015	.019
6. Grapefruit	May 26	5.70	.015	.028	0.38	.006	1.34	.020	.026	0.38	.006	1.41	.021	.027
7. Valencia orange	June 4	5.25	0.022	0.038	0.26	0.004	2.08	0.031	0.035	0.26	0.004	2.14	0.032	0.036

TABLE 2

ETHER-SOLUBLE ORGANIC ACIDS OF DRIED PEEL OF DIFFERENT VARIETIES OF CITRUS

SAMPLE NO. AND VARIETY	MOISTURE (%)	pH OF SAP	ETHER-SOLUBLE ORGANIC ACIDS									
			Total acids, as citric (pH 7.8–2.6)		Citric acid		Malic acid		Oxalic acid		Total citric, malic, and oxalic acids	
			mg./gm.*	me./gm.†	mg./gm.	me./gm.	mg./gm.	me./gm.	mg./gm.	me./gm.	mg./gm.*	me./gm.
1. Navel orange	73.02	5.00	4.93	0.077	0.38	0.006	2.61	0.039	1.13	0.025	4.48	0.070
2. Navel orange	73.51	5.05	5.12	.080	.51	.008	2.88	.043	1.26	.028	5.06	.079
3. Navel orange	76.07	5.40	5.32	.083	.51	.008	2.21	.033	1.80	.040	5.19	.081
4. Navel orange	74.80	5.30	3.07	.048	.32	.005	1.41	.021	0.95	.021	3.01	.047
5. Valencia orange	77.24	‡	3.14	.049	.26	.004	1.61	.024	0.90	.020	3.07	.048
6. Valencia orange	74.95	‡	3.33	.052	.32	.005	1.54	.023	1.08	.024	3.33	.052
7. Valencia orange	73.56	‡	3.97	.062	.32	.005	2.28	.034	0.99	.022	3.91	.061
8. Valencia orange	75.11	5.25	3.84	.060	.32	.005	2.01	.030	1.08	.024	3.78	.059
9. Grapefruit	80.18	‡	2.88	.045	.32	.005	1.27	.019	0.86	.019	2.75	.043
10. Grapefruit	80.56	‡	2.75	.043	.32	.005	1.47	.022	0.68	.015	2.69	.042
11. Grapefruit	82.39	‡	2.37	.037	.38	.006	1.34	.020	0.50	.011	2.37	.037
12. Grapefruit	81.52	5.65	2.88	.045	.38	.006	1.68	.025	0.59	.013	2.82	.044
13. Lemon	83.59	5.38	3.59	.056	.26	.004	1.27	.019	1.40	.031	3.46	.054
14. Lemon	83.54	5.48	3.14	.049	.38	.006	1.07	.016	1.40	.031	3.39	.053
15. Lemon	84.24	5.53	2.18	.034	.32	.005	0.94	.014	0.63	.014	2.11	.033
16. Lemon	85.04	‡	2.88	.045	.32	.005	1.34	.020	0.86	.019	2.82	.044
17. Lemon	83.92	‡	2.43	0.038	0.19	0.003	1.27	0.019	0.63	0.014	2.31	0.036

* Milligrams of acid (as citric) per gram of fresh peel.

† Milliequivalent per gram of fresh peel.

‡ Data undetermined.

acids in grapefruit peel is reflected in the higher pH of its peel sap.

The distinctive features of table 2 are the comparatively low concentration of citric acids and the relatively high concentrations of malic and oxalic acids in the various peel samples. By extracting dried peel with ether at pH 1, the insoluble oxalates were made soluble and were readily removed from the tissue by the solvent. The abundance of calcium in the peel (table 3) indicates that any oxalic acid occurring in the peel would be immediately converted to the insoluble calcium salt. This is, of course, true only if an excess of calcium is present in the peel over that required to form calcium pectate. The fact that only a trace of oxalic acid appeared in the peel sap is evidence that sufficient calcium was present to convert the oxalic acid to insoluble calcium oxalate. The high pH of the peel sap is also evidence that the oxalic acid is in the salt form. These data substantiate the observations of BARTHOLOMEW and REED (2), who have noted the presence of calcium oxalate crystals in the peels of citrus fruits; and of PIERCE and APPLEMAN (5), who have shown that plants high in oxalic acid contained only traces of sap-soluble calcium. The high calcium content of peel (table 3), in comparison with that of pulp and juice, is certainly highly correlated with the amount of insoluble oxalate. In fact, calcium and magnesium are the only inorganic cations which exist in the peel in concentrations sufficiently high to form crystalline insoluble salts with the organic acids.

Calcium in the peel may react with other chemical substances in the sap besides oxalic acid. As suggested previously, amorphous precipitates such as calcium pectate may occur as the result of reaction of calcium with pectin. Undoubtedly, the inorganic cations precipitate, at various rates, the hydrated colloids of the peel. A protective action is exerted by many organic colloids to prevent the precipitation of compounds that have exceeded their solubility product in pure solution.

The exceptionally low concentration of organic acids in the peel of citrus fruits, in comparison with that in the pulp, strongly suggests that the location of organic acid synthesis is in the pulp in citrus fruits, according to the following chain of reasoning: Citrus fruits, unlike pomaceous fruits, have no fleshy centers that are permeated by vascular bundles. The vascular system is chiefly confined to the mesocarp (spongy parenchyma of the peel), which therefore serves the important function of trans-

TABLE 3

RELATIVE AMOUNTS OF CALCIUM AND MAGNESIUM IN PEEL, PULP, AND JUICE OF ORANGES AND GRAPEFRUIT

VARIETY	MOISTURE (% FRESH WEIGHT)			CALCIUM (% DRY WEIGHT)			MAGNESIUM (% DRY WEIGHT)		
	Peel	Pulp	Juice	Peel	Pulp	Juice	Peel	Pulp	Juice
Valencia orange	71.0	84.9	87.2	0.76	0.30	0.06	0.12	0.08	0.09
Navel orange	74.0	85.7	88.1	.56	.31	.06	.10	.09	.11
Grapefruit	80.3	87.3	89.1	0.64	0.26	0.07	0.10	0.10	0.11

porting water and solutes from the tree to the pulp vesicles. The experiments of HODGSON (4) and of BARTHOLOMEW (1) showed the existence of an equilibrium between the water in citrus trees and their fruits. This equilibrium is manifested by the diurnal changes in volume of fruits on trees subjected to an environment conducive to excessive transpiration from leaves. The results of these studies led REED (8) to conclude that the translocation of liquids in citrus fruits is through the layers of hydrophilic colloids on the cell walls of the mesocarp and that the juice vesicles in the pulp exert sufficient suction pressure to pull water from the hydrated colloids.

If the organic acids are synthesized in the leaves and subsequently transferred to the juice vesicles through the albedo (mesocarp), it would appear that higher concentrations of these acids than are present should occur in the mesocarp. The latter contains sufficient cations to form salts (soluble and insoluble) if the organic acids pass through the peel to the vesicles, which contain large amounts of organic acids. On the contrary, however, the mesocarp is notably low in amounts of free and combined organic acids. During the entire growth and maturation of the fruit, the passage of water and solutes from leaves to vesicles, and from vesicles to leaves during excessive leaf transpiration, did not result in accumulation of organic acids in the peel of mature fruits. The lack of accumulation of organic acids under these conditions is highly suggestive that the organic acids are synthesized in the vesicles and not in the leaves. This deduction agrees with the limited experiments of RICEVUTO (9), who concluded that citric acid is formed in lemon fruits from reducing sugars and pentosans by enzymatic action.

TITRATION CURVES OF PEEL SAPS

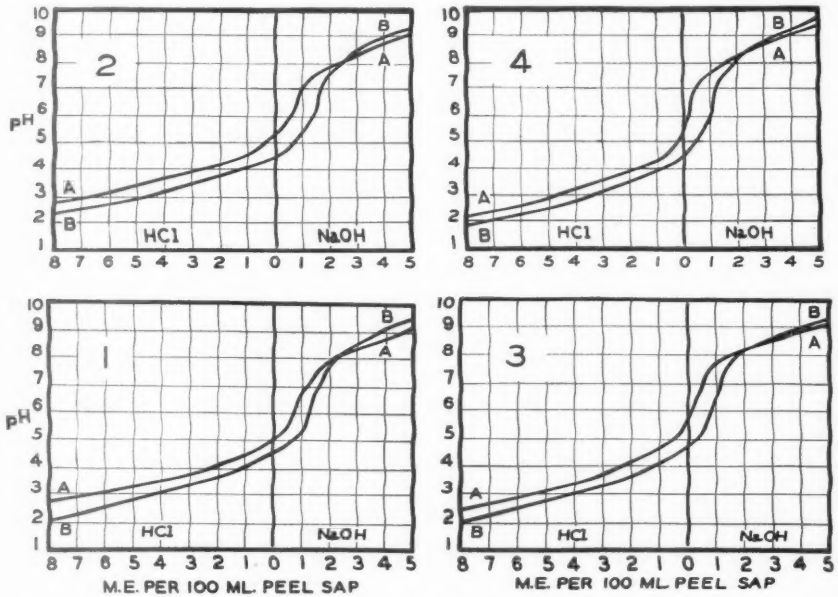
The relation of pH to the acid constituents of peel sap can be further elucidated by following the resistance to changes in pH upon addition of increments of standard acid or alkali to a known amount of sap. Peel sap, like all plant extracts, is to a varying degree capable of resisting changes in pH upon the addition of strong acids or bases. It was therefore decided to determine experimentally what substances were responsible for the buffer effect of the sap.

It was important to determine the effect of the soluble pectins in the centrifuged sap on the shapes of the buffer curves. The titration curves of normal sap (curves *A*) and of sap from which pectins had been removed (curves *B*) are shown for navel and Valencia oranges, grapefruit, and lemons in figures 1–4, respectively. Although the two curves in each figure are nearly parallel, they are distinctly different. The alcohol used for precipitating pectins probably precipitated some of the soluble buffer salts also and thus caused an increase in acidity and lowered the position of curve *B* on the graph. The amounts of free acid in these saps are represented by those portions of the curves between zero and the milliequivalents of NaOH required to bring the system to a pH of 8.2. It can be seen that the concentration of free acid, organic and inorganic, is relatively small in these saps. The total-acid radical calculated from the titration curves between pH 7.8 and 2.6 was greater than the values determined from similar titrations of the ether-soluble organic acids extracted from the sap.

The position of the curves on the graphs, with respect to buffer properties, is influenced by the concentration of free acid in the sap. In other words, the buffer capacity of a solution is governed by the

concentration of the components. As citrus-peel sap is not highly buffered, titration curves of saps of different dilutions would have different slopes. Titration curves of peel saps of different varieties of citrus are very similar, and all of them are located at nearly the same position on the graphs. As shown by the zero points on curves *A* and *B* (figs. 3, 4), the pH of the natural saps from grapefruit and lemon peels are somewhat higher than corresponding values for peels of navel and Valencia oranges.

The titration curves of the various peel saps are vastly different from those of the corresponding juices of the pulp. The peel of citrus fruits contains a comparatively small amount of organic acids and their salts. In the absence of these constituents, the pH values of the peels are high, and these systems consequently have only a slight buffer capacity. The pulp juices of citrus fruits, on the other hand, contain larger amounts of organic acids and their salts and have lower pH values and higher buffer capacities.



FIGS. 1–4.—Titration curves of peel sap. *A*, normal; *B*, pectin removed. *Fig. 1*, navel orange. *Fig. 2*, Valencia orange. Fig. 3, grapefruit. Fig. 4, lemon.

Summary

1. The concentrations of ether-soluble organic acids are much lower in peels of citrus fruits (oranges, grapefruit, and lemons) than in juice samples of corresponding pulps. The high pH values of the peel saps indicate that most of the organic acids are present in the salt form and not in the free state. The amounts of citric and malic acids in the expressed saps agreed satisfactorily with the total organic acids titrated between pH 7.8 and 2.6. The organic acids precipitated from the peel sap with lead acetate

agreed with the total organic acids titrated between pH 7.8 and 2.6 on a water solution of the ether extract. The citric and malic acids values of the ether extract agreed substantially with similar values of the lead acetate precipitate. The ether liquid extraction method is satisfactory for the isolation of organic acids from citrus-peel saps.

2. The total nonvolatile organic acids were extracted from dried peel of citrus fruits with absolute ether at pH 1. The organic acid content consisted chiefly of citric, malic, and oxalic acids. The citric acid content is very low in comparison with the concentrations of malic and oxalic acids. The oxalic acid is present in the peel as insoluble calcium oxalate, with the exception of a trace occurring in the peel sap.

3. The titration curves of peel saps of citrus fruits (navel and Valencia oranges, grapefruit, and lemons) show that these systems contain comparatively small amounts of organic acids and their salts. In the absence of these constituents, the pH values of the peels are high, and these systems consequently have only a slight buffer capacity.

UNIVERSITY OF CALIFORNIA
CITRUS EXPERIMENT STATION
RIVERSIDE, CALIFORNIA

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SURVEY OF HIGHER PLANTS FOR PRESENCE OF ANTIBACTERIAL SUBSTANCES

LESLEY E. HAYES

Introduction

The fact that substances with antibacterial properties have been found in a number of species of nongreen plants led to the supposition that similar substances occur in green plants. Although a large number of species of higher plants have been investigated for the presence of such compounds by previous workers, their work by no means exhausted the possibilities in this line of research.

Demonstration by *in vitro* methods of antibacterial properties in a substance synthesized by plants does not prove that compound to be of therapeutic value. However, *in vitro* techniques do provide a rapid method for detecting antibacterial substances which may warrant more intensive investigation. It was the purpose of this investigation to make *in vitro* tests for antibacterial activity of extracts of tissues of higher plants common to Ohio.

Review of literature

OSBORN (8), an English worker, has reported the results of an experimental survey on 2300 species of higher plants and has shown that several species in the Ranunculaceae, Cruciferae, Compositae, and Liliaceae contain substances that inhibit growth of *Staphylococcus aureus* and *Escherichia coli*.

PRATT *et al.* (9) obtained an antibiotic—chlorellin—from the culture solution and the cell mass of cultures of *Chlorella vulgaris* and *C. pyrenoidosa*. In solutions of 0.03–0.1 mg. chlorellin per cc. of water, the antibiotic caused a zone of inhibition 45 mm. wide on *S. aureus* plates.

HEATLEY (6), another English worker, reported a comprehensive study of crepin, an antibiotic obtained from the buds and flowers of *Crepis taraxacifolia*. The substance is thermostable, is soluble in pyridine and alcohol, and has a molecular weight between 236 and 275. The suggested molecular formula for crepin is $C_{14}H_{16}O_4$. It inhibits growth of certain gram-positive and gram-negative bacteria, and its inhibitory activity is enhanced by treatment with human serum for 24 hours at 37° C. It is slightly toxic to leucocytes.

SANDERS *et al.* (12), working in Indiana, tested 120 plant species, 22 of which showed varying degrees of bacterial inhibition. Juice was expressed, without use of solvents, in a Carver hydraulic press and tested against *S. aureus* and *Bacillus subtilis*.

SEEGAL and HOLDEN (13), at the University of Illinois, described activity of extracts of *Anemone pulsatilla* and a species of buttercup (name not given) against *Mycobacterium tuberculosis hominis* and *Monilia albicans*. They prepared the samples in two ways, either by steam distillation or by autoclaving an extract made from one part water to one part ground plant material. The extract was found to be toxic to laboratory animals, but the report did not state whether the extract was administered orally or parenterally.

In China, CHEN *et al.* (4) found a principle in *Eleocharis tuberosa* which was active against *S. aureus*, *Escherichia coli*,

and *B. subtilis*. This substance, which they called puchiin, is thermolabile and is soluble in water but not in ether, petroleum ether, chloroform, or benzene.

LUCAS and LEWIS (7), working in Michigan, reported antibacterial activity of water extracts of *Lonicera tartarica*, *Vaccinium corymbosum*, *Sorbus americana*, *Verbascum thapsus*, and *Paeonia officinalis*. The writer's tests on *P. officinalis* showed stimulation of bacterial growth rather than inhibition.

CAVALLITO *et al.* (2, 3) determined methods of isolation, physical properties, and antibacterial action of inhibitors found in *Arctium minus* and *Allium sativum*.

Investigation of antibiotic activity of higher plants has not been limited to antibacterial effects. TOKIN (10), a Russian worker, reported discovery of "phytoncides," the name applied to volatile substances, produced by plants, which are strongly bactericidal and protistocidal. These vapors are also lethal to yeast and to eggs of mollusks and frogs. Phytoncides have been found in roots of onion, garlic, and *Paeonia anomalia*. TOROPTSEV and FILATOVA (11), also from Russia, reported that the vapors of onion and garlic were effective in stimulating regeneration of tissue in both aseptic and infected wounds.

Material and methods

Tests were made on 231 plant species collected in Franklin, Hocking, Scioto, Pike, and Adams counties, Ohio. As a rule the determinations were carried out immediately or within 24 hours. When not used immediately, the specimens were kept wrapped in moist paper at 8° C. A few species previously tested by other workers were included because of the premise that antibacterial activity of plant extracts may vary with stage in growth of the plant or with method of testing employed. Certain species tested —ginseng, heal-all, joe-pye weed, and ninebark—were of interest because folklore attributes medicinal value to them.

GRAY (5) was used as the authority in nomenclature of species except for the Bryophytes, some of the Pteridophytes, and a few horticultural varieties.

The extracts were made by grinding the specimens in a meat-chopper and then macerating them in a Waring Blendor with from one-half to three times their weight of distilled water for 5 minutes. The amount of water added depended on the succulence of the plant. The macerated material was then filtered in a Buchner funnel under suction, using two disks of no. 1 filter paper.

The activity of plant extracts was tested by a method similar to that devised by ABRAHAM *et al.* (1) in connection with penicillin assay. Tryptose-dextrose agar was used as the culture medium in the tests. The medium was made according to the following formula:

Bacto-tryptose	20.0 gm.
Bacto-dextrose	2.0 gm.
Di-sodium phosphate	2.5 gm.
Sodium chloride	5.0 gm.
Powdered agar	15.0 gm.
Distilled water	1000.0 ml.

Plates were poured to a depth of approximately 3 mm. of agar, and care was taken to keep the plates on a level surface until the agar had solidified.

Nutrient broth, used as an inoculating medium, was prepared as follows:

Beef extract	3 gm.
Peptone	5 gm.
Sodium chloride	5 gm.
Distilled water	1000 ml.

The test organisms used were *Staphylococcus aureus*, *Escherichia coli*, *Erwinia carotovora*, and *Phytomonas tumefaciens*. The first two organisms are

TABLE 1

RESULTS OF TESTS FOR ANTIBACTERIAL ACTION OF EXTRACTS OF EIGHTEEN SPECIES OF GREEN PLANTS

. . . . = No activity
S = Marked stimulation
+ = Inhibitory zone, 5 mm.
++ = Inhibitory zone, 6–10 mm.
+++ = Inhibitory zone, 11–15 mm.
++++ = Inhibitory zone, 16–25 mm.
+++++ = Inhibitory zone, 26–50 mm.

Species	Staphylococcus aureus	Escherichia coli	Erwinia carotovora	Phytomonas tumefaciens
Allium cernuum				
Leaves	++++	+	++	+++
Leaf, stem, flower	S	. . . .	+	. . . .
Bulbs	S	. . . .	. . . .	++
A. tricoccum				
Leaves	++++	++	++++	++++
A. vineale				
Leaf	+++	++++	+++	+++
Leaf, stem, flower	SS	S	+	. . . .
Bulbs	++++	+	++	++++
Bulbs (2d sample)	++++	+	++	++++
Asarum canadense				
Leaf, stem, flower	++++	. . . .	. . . .	. . . .
Leaf, stem, root	++	. . . .	. . . .	. . . .
Leaf, stem, root (boiled 5 min.)	++	. . . .	. . . .	. . . .
Barbarea vulgaris				
Winter rosette	++	S	+	++
Entire plant (summer)	S	S	S	S
Summer rosette	. . . .	. . . .	. . . .	. . . .
Berberis thunbergii				
Fruit	+++	+++	++	+
Fruit (2d sample)	+++	+++	++	+
Flowers	S	S	+	. . . .
Celastrus scandens				
Fruit	+	+++	++	+
Convolvulus arvensis				
Leaf, stem	. . . .	. . . .	++++	. . . .
Root	. . . .	. . . .	++++	++
Leaf, stem	. . . .	. . . .	++++	?
Crataegus sp.				
Fruit	++	+	+	+
Erythronium americanum				
Leaves, flowers	+	+	++	. . . .
Ilex decidua				
Fruit	+	++	+++	. . . .
Fruit (2d sample)	+	++	+++	. . . .
Lepidium draba				
All but root	+++	+++	+	+
Entire plant	++	++	+	++
Medeola virginiana				
Leaf, stem, root	+	. . . .	++	S

TABLE 1—*Continued*

Species	Staphylococcus aureus	Escherichia coli	Erwinia carotovora	Phytomonas tumefaciens
Oenothera biennis				
Winter rosette		+++		
Summer leaf, stem, root				
Ranunculus abortivus				
All but root	+++		+++	
Entire plant	+++++	+++++		
Entire plant (2d sample)	+++++	+++++		
Sanguinaria canadensis				
Entire plant	+++	S		
Leaf, stem, rhizome	++			
Leaf, stem, rhizome (extract boiled 5 min.)	++			
Dried rhizome	++			
Taxus canadensis				
Fruit	+++	+++	++++	
Fruit (boiled 5 min.)	+++	+++	++++	
Leaves	S		S	
Verbascum thapsus				
Leaves (after flowering)				
Spring rosette			++	+
Spring rosette and root			++	+

sometimes pathogenic to man, and the last two are pathogenic to plants. *S. aureus* is gram-positive, the other three are gram-negative.

The agar plates were inoculated by flooding with 24-hour nutrient-broth cultures of the test organism and allowed to drain inverted on a flat board sterilized with phenol solution.

Sterile cylinders (made from 10 mm. lengths of glass tubing, 15 mm. in diameter, with one end beveled toward the inside) were placed on the inoculated agar with slight pressure, making a water- and bacteria-tight seal. Approximately 0.5 ml. of extract to be tested was introduced into this cylinder. Throughout the procedure the usual precautions were taken to prevent contamination. One to six samples per plate could be tested. Plates of *Staphylococcus aureus* and *Escherichia coli* were incubated 16–18 hours at 37° C., and plates of the plant pathogens were incubated 18–24 hours at room temperature (approximately 27° C.). The tests were carried out in triplicate, and results were evaluated by measuring zones of inhibition or stimulation of bacterial growth.

Results and discussion

Some of the extracts markedly inhibited bacterial growth, some caused increased bacterial growth, and others showed no activity. Of the 231 species tested, extracts from 46 inhibited growth of one or more species of the test organisms. The most marked inhibition was obtained in the tests of the species listed in table 1.

The following 213 species showed little or no inhibition of bacterial growth:

Acanthopyrus sp.
Achillea millefolium
Acorus calamus
Adiantum pedatum
Aesculus hippocastanum
Agrimonia sp.
Agrostemma githago
Ambrosia artemisiifolia
A. trifida

Anemone canadensis
Antennaria parlinii
A. plantaginifolia
Apocynum cannabinum
Arabis hirsuta
Aralia spinosa
Arisaema triphyllum
Aruncus sylvester
Asclepias syriaca
Asclepiodora viridis
Asimina triloba
Asparagus officinalis
Aspidium marginale
Belamcanda chinensis
Benzoin aestivale
Blephilia ciliata
Botrychium virginianum
Brassica nigra
Buchloë dactyloides
Camptosorus rhizophyllus
Capsella bursa-pastoris
Cardamine bulbosa
C. pennsylvanica
Carduus crispus
Carex stricta
Castalia tuberosa
Castilleja coccinea
Caulophyllum thalictroides
Ceratophyllum demersum
Chaerophyllum procumbens
Chara foetida
Chenopodium album
Chimaphila maculata
Chrysanthemum leucanthemum
Cibotium schiedei
Cichorium intybus
Cicuta bulbifera
C. maculata
Cimicifuga racemosa
Cirsium arvense
Cladonia rangiferina
Conocephalum conicum
Crepis biennis
Crocus vernus
Cunila origanoides
Cuscuta sp.
Cynoglossum virginianum
Cyperus papyrus
Cystopteris fragilis
Dactylis glomerata
Daucus carota
Decodon verticillatus
Dentaria laciniata
Dianthera americana
Dicentra canadensis
Diospyros virginiana
Dipsacus sylvestris
Dodecatheon meadia
Eleusine indica
Epipactis tesselata
Equisetum arvense
Eragrostis cilianensis
Erigeron philadelphicus
Eupatorium purpureum
Euphorbia corollata
Evonymus obovatus
Galinsoga parviflora
Galium aparine
Gaultheria procumbens
Geranium maculatum
Geum vernum
Ginkgo biloba
Gillenia stipulata
Hedera helix
Heracleum lanatum
Heuchera sanguinea
H. villosa
Hibiscus moscheutos
Hieracium venosum
Houstonia caerulea
H. longifolia
Hydrophyllum appendiculatum
Hypericum perforatum
Ilex opaca
Impatiens pallida
Iodanthus pinnatifidus
Iris germanica
Juniperus horizontalis
Krigia amplexicaulis
Lactuca canadensis
Lamium purpureum
Laportea canadensis
Leonurus cardiaca
Lepachys pinnata
Lepidium campestre
L. virginicum
Liriodendron tulipifera
Lithospermum arvense
L. canescens
Lychnis alba
Lycopodium lucidulum
Lygodium japonicum
Lysimachia nummularia
L. quadrifolia
Malva rotundifolia
Medicago lupulina
Melilotus alba
Menyanthes trifoliata
Nepeta hederacea
Nephrolepis cordifolia
N. exaltata *var.* bostoniensis
Oenothera fruticosa
Onoclea sensibilis
Osmorhiza claytoni
O. longistylis
Osmunda regalis
Oxalis stricta
Paeonia officinalis
Panax quinquefolium
Pastinaca sativa
Pedicularis canadensis
Pentstemon laevigatus
Phacelia purshii
Phlox divaricata
Physocarpus opulifolius
Picea abies
Pinus strobus
Plantago lanceolata
P. major
Platanus occidentalis
Poa pratensis
Podophyllum peltatum
Polemonium reptans
Polygonatum biflorum
Polygonum amphibium
Polypodium aureum
P. longifolium
P. polypodioides
P. vulgare
Polystichum acrostichoides
P. lonchitis
Potamogeton crispus
P. natans
P. pectinatus
Potentilla canadensis
P. recta
Prunella vulgaris
Prunus persica
Psilotum nudum
Psoralea pedunculata
Pteris aquilina
Ranunculus septentrionalis
Rhus canadensis
Ruellia ciliosa
Rumex acetosella
R. crispus
Sagittaria latifolia
Salvia lyrata
Salvinia natans
Sambucus canadensis
Saponaria officinalis
Sarracenia purpurea
Sedum stoloniferum

Selaginella braunii
S. kraussiana
Senecio aureus
Sida hermaphrodita
Silene virginica
Silphium perfoliatum
S. terebinthinaceum
Smilacina racemosa
S. stellata
Solanum carolinense
S. dulcamara
Spartina michauxiana
Specularia perfoliata
Stellaria media
Symphoricarpos orbiculatus
Symplocarpus foetidus
Taraxacum officinale
Thalictrum dioicum
Tillandsia usneoides
Tragopogon pratensis
Trifolium hybridum
T. pratense
T. repens
Trillium sessile
Typha angustifolia
Umbilicaria delineata
Uvularia perfoliata
Valeriana pauciflora
Valerianella radiata
Verbena angustifolia
Veronica arvensis
V. officinalis
Viburnum opulus
Vicia cracca
Viola papilionacea
Vitis labrusca
Vinca minor
Yucca filamentosa
Zebrina pendula
Zizia aurea

The inhibitory activity of plant tissues varied with (*a*) species of bacteria tested, (*b*) part of plant used, (*c*) stage in which plant was collected, and (*d*) temperature under which plant was growing at time of collection. Other variations in inhibitory activity may be due to (*e*) method of extraction, (*f*) culture medium used, and (*g*) diffusibility of the extract through agar.

The antibacterial substances found in *Asarum canadense*, *Sanguinaria canadensis*, and *Taxus canadensis* were found to be thermostable. The antibacterial substance in *S. canadensis* was not lost upon drying.

Summary

Water extracts of 231 species of higher plants were tested for antibacterial properties against *Staphylococcus aureus*, *Escherichia coli*, *Erwinia carotovora*, and *Phytomonas tumefaciens*. Eighteen of the species showed sufficiently high antibacterial effects against one or more of the organisms to warrant their inclusion in table 1.

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CISNE, ILLINOIS

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SELECTIVE HERBICIDAL EFFECTS OF 2,4-DICHLOROPHENOXYACETIC ACID APPLIED TO TURF IN DRY MIXTURES WITH FERTILIZER

PAUL C. MARTH[1] AND JOHN W. MITCHELL[2]

Introduction

2,4-Dichlorophenoxyacetic acid (2,4-D) is effective as a herbicide when applied as a dust to the foliage of various kinds of weeds (**2**) or to soil in which these plants are growing (**1, 6, 7, 8**). In general, most lawn grasses, such as bluegrass (*Poa pratensis*), red top (*Agrostis alba*), and red fescue (*Festuca rubra*), have proved to be relatively resistant to 2,4-D but have shown, in varying degrees, a temporary checking in their rate of growth following use of the sprays (**5**). Certain grasses, such as the bents, have proved to be much more sensitive and have exhibited marked injury with applications that ordinarily have but a slight effect on bluegrass. To speed up recovery of grasses from this initial growth-depressing effect of the herbicide, it was found helpful to supply nutrient material, such as urea, to them at the time of treatment (**3**). Spray application of a water mixture of urea (supplying 60 lb. per acre) and 2,4-D (1.5 lb. per acre) had beneficial effects on recovery of bluegrass. Preliminary experiments with dry application of complete fertilizers containing various amounts of 2,4-D have also given similar effects (**4**). The present experiments were undertaken to determine (*a*) the amounts of 2,4-D applied dry in mixture with complete fertilizer that would be required per acre selectively to eradicate weeds from turf and (*b*) the effect of such mixtures on the growth of turf from applications at various seasons of the year.

[1] Physiologist, [2] Senior Physiologist, Bureau of Plant Industry, Soils and Agricultural Engineering, Agricultural Research Administration, U.S. Department of Agriculture, Beltsville, Md.

Methods

2,4-Dichlorophenoxyacetic acid was used throughout these experiments, except where the sodium, ammonium, or morpholine salts of the acid were substituted for comparative purposes. The mineral fertilizer was of commercial grade, 10–6–4 (N, P_2O_5, K_2O) analysis, purchased on the open market, and com-

monly used for fertilizing lawns and gardens.

A mechanically operated barrel-type concrete mixer was used in preparing the fertilizer plus 2,4-D mixtures. The desired weighed amount of 2,4-D was first mixed thoroughly by hand with 1 lb. of fertilizer prior to adding this mixture to the desired weighed amount of fertilizer (usually about 20 lb.) as it revolved in the mixer, and the mixing operation was then continued for a period of 10 minutes.

All fertilizer treatments were at the rate of 600 lb. per acre, the amount generally recommended for stimulating the growth and development of sod. All mixtures were applied on the same or succeeding day after mixing.

The area of established sod selected for experimentation was composed mainly of Kentucky bluegrass, red top, and fescue grasses heavily and uniformly infested with narrow-leaved plantain (*Plantago lanceolata*). Other weedy species present included sheep sorrel (*Rumex acetosella*), black medic (*Medicago lupulina*), and white clover (*Trifolium repens*). Two sets of plots, thirty each, were marked off; treatments on one set were applied on November 6, 1945; on the other, on March 22, 1946. Individual plots were 2 feet wide and 25 feet long, and a 1-foot border was left between plots. At each date of treatment the thirty plots were divided into five replicates, and the six different treatments were assigned at random to plots within blocks. The applications consisted of 0, 1.5, 3, 6, and 9 lb. per acre of 2,4-D mixed with fertilizer and of an unfertilized control that received no 2,4-D. The weighed amount of fertilizer mixture was applied as carefully and evenly as possible to each plot by hand when the foliage was free of surface moisture.

In another experiment on larger areas ($\frac{1}{6}$–1 acre) a fertilizer attachment on a 12-foot lawn drill drawn by a tractor was used. Sufficient 2,4-D (acid) was mixed with fertilizer by hand-shoveling to deliver 4 lb. of 2,4-D in 300 lb. of fertilizer per acre; this was applied on March 15, 1946, to a rectangular lawn area approximately 1 acre in size. Again on March 26 the same distributor was used in treating three adjacent $\frac{1}{6}$-acre lawn areas with 4 lb. per acre of the morpholine, sodium, and ammonium salts of 2,4-D, respectively. Since the area had already been fertilized with 300 lb. per acre of 10–6–4 fertilizer, the various salts were applied in mixture with fine sand.

Clippings were obtained from all plots by use of a small power mower adjusted to cut at a height of 1.5 inches and fitted with a grass-catcher. Fresh weights of both grass and weed foliage were obtained after the clippings had been hand-sorted.

Results

LATE FALL APPLICATION.—On April 4, 1946, there was no significant difference in the growth of grass between the fertilized control plots (no 2,4-D) and others that received varying amounts of 2,4-D with fertilizer on the preceding November 6 (table 1). In comparison with the fertilized control plot, however, the addition of both 6 and 9 lb. per acre of 2,4-D caused a marked intensification of green color in the grass foliage so that these plots, by visual inspection, could very readily be selected from among the various treatments. 2,4-D applications of 1.5 and 3 lb. per acre also caused greening of the grass but to a lesser degree. This difference in coloration persisted until about June 1.

From the standpoint of weed eradica-

tion even the smallest amount of 2,4-D (1.5 lb. per acre) applied in the dry form together with fertilizer gave approximately 100% control of narrow-leaved plantain. At the 6- and 9-lb. rates the small amount of weed clippings obtained consisted almost entirely of sheep sorrel, a plant with underground stolons that has sometimes been found difficult to control by means of the usual 2,4-D spray treatment.

TABLE 1

EFFECT OF FALL APPLICATIONS OF 2,4,-D IN DRY MIXTURE WITH COMMERCIAL (10–6–4) FERTILIZER ON GROWTH OF GRASS AND DESTRUCTION OF WEEDS IN PLOTS OF 50 SQUARE FEET TREATED NOVEMBER 6, 1945. CLIPPINGS OBTAINED APRIL 4, 1946

TREATMENT		FRESH CLIPPING WEIGHTS (AVERAGE OF 5 PLOTS)		PERCENTAGE OF WEEDS
2,4-D (lb./acre)	10–6–4 fertilizer (lb./acre)	(Grass) (gm.)	Weeds (gm.)	
0.....	0	145.4*	28.6	16.9
0.....	600	329.0	62.2	16.5
1.5.....	600	332.6	7.4	2.5
3.0.....	600	343.0	6.0	1.4
6.0.....	600	338.0	2.4	0.7
9.0.....	600	337.4	1.8	0.5

* Differences of 78.6 gm. are significant at the 5% level.

EFFECT ON GRASS SEED PRODUCTION.—After the grass had developed following clipping on April 4, differences in the number of seed stalks of grass on the different plots became apparent. The seed heads, which consisted almost entirely of bluegrass, were permitted to develop fully, and on June 6, 1946, the bluegrass seed heads and straw were harvested by individual plots. At this time the straw had just begun to wither, and the seeds were judged to have reached maturity. After drying for 2 weeks in a greenhouse at approximately 70°–100° F., the seed heads were further dried overnight in a ventilated oven at 100° F.; the seeds were then rubbed out by hand and weighed.

Although the results on seed production are not sufficient for final conclusions, it is of interest that the average seed (plus chaff) production per plot amounted to 4.2, 17.0, 23.5, 22.1, 17.5, and 22.5 gm., respectively, for the control, fertilized control, and fertilizer plus 1.5, 3, 6, or 9 lb. of 2,4-D per acre. From the standpoint of grass seed production the significance of these results lies principally in the fact that the plots that received 2,4-D treatment were free of weeds and therefore relatively free of weed seeds. Further experiments are in progress to determine the possible effects of comparable applications of 2,4-D to soil on the yield and viability of grass seeds.

SPRING APPLICATION.—Marked differences in the growth of grass were obtained from dry applications on March 22 of fertilizer plus 2,4-D mixtures (table 2). On both the May 6 and the July 9 clipping dates plots that had received 1.5 lb. of 2,4-D per acre together with fertilizer produced a significantly greater amount of grass clippings than did plots that received fertilizer alone. When 2,4-D was applied at 3 and 6 lb. per acre, the weights of grass clippings were slightly greater than in the controls (fertilized). At the first clipping these latter differences were not statistically significant, but at the later clipping (July 9) they were found to be significant. Larger amounts of 2,4-D were injurious to the growth of grass (table 2). The reduction of 182.2 gm. in average clipping weight of grass on May 6 caused by 9 lb. of 2,4-D per acre in comparison with the fertilized control was found to be highly significant. By July 9, however, the grass

had recovered so that there was no significant difference between the weights of clippings from the fertilized control and from plots treated with 9 lb. of 2,4-D per acre. The injurious effects of 2,4-D applied at the rate of 9 lb. per acre were clearly evident in the appearance of the grass, the blades being narrower and shorter, although deeper green in color.

As with fall application, an intensification of green coloration of the grass foliage was noted at the 3-, 6-, and 9-lb. rates of 2,4-D applications in spring, in comparison with the fertilized control plots.

The weed-killing effects of 2,4-D were equally effective in the fall and spring applications under the condition of these experiments (fig. 1).

TREATMENT OF LARGE LAWN AREAS.—The feasibility of applying dry 2,4-D mixtures on a larger scale for selective herbicidal effects was tested during the spring of 1946. All the treatments, with

TABLE 2

EFFECT OF SPRING APPLICATION OF 2,4-D IN DRY MIXTURE WITH COMMERCIAL (10–6–4) FERTILIZER ON SUBSEQUENT YIELD OF GRASS AND WEED CLIPPINGS IN PLOTS OF 50 SQUARE FEET TREATED MARCH 22, 1946

TREATMENT		FRESH CLIPPING WEIGHTS (AVERAGE OF 5 PLOTS)					
		May 6, 1946			July 9, 1946		
2,4-D (lb./acre)	10–6–4 fertilizer (lb./acre)	Grass (gm.)	Weeds (gm.)	Percentage of weeds	Grass (gm.)	Weeds (gm.)	Percentage of weeds
0	0	366.4*	53.6	11.4	304.4†	92.5	23.2
0	600	749.6	182.4	21.5	384.8	166.8	30.1
1.5	600	931.8	12.2	1.1	530.8	24.2	4.2
3.0	600	805.8	6.2	0.7	485.2	10.6	2.0
6.0	600	756.2	1.8	0.2	427.2	8.9	2.0
9.0	600	560.6	1.4	0.2	380.8	9.4	2.4

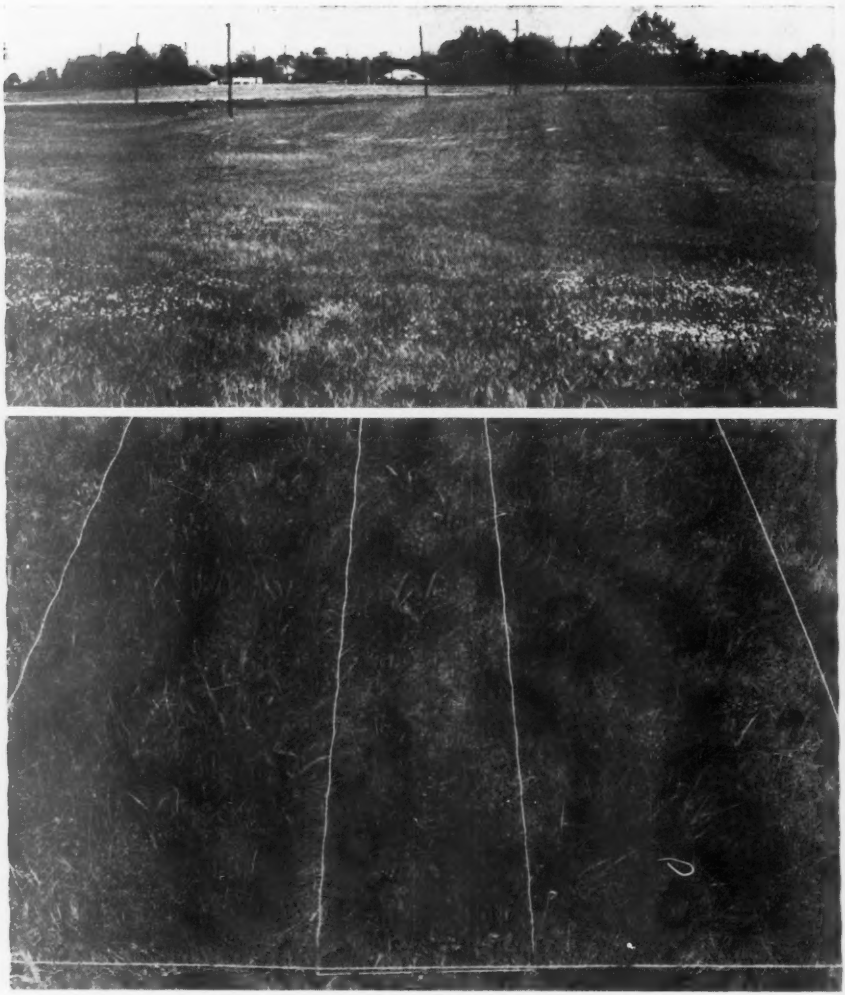
* Differences of 146.0 gm. are significant at the 5% level.

† Differences of 69.3 gm. are significant at the 5% level.

TABLE 3

HERBICIDAL EFFECTS OF VARIOUS SALTS AND THE ACID FORM OF 2,4-D APPLIED AS DRY MIXTURES AT RATE OF 4 LB. PER ACRE TO GRASS SOD

TREATMENT	PERCENTAGE OF CLIPPINGS THAT CONSISTED OF WEED FOLIAGE					
	Sample no.					Average
	1	2	3	4	5	
Control	60.0	54.4	25.2	56.4	46.0	48.4
2,4-D acid	10.2	13.6	12.8	14.0	10.4	12.2
2,4-D ammonium salt	1.0	1.6	1.2	4.0	1.6	1.9
2,4-D sodium salt	14.4	11.2	10.8	16.0	12.4	13.0
2,4-D morpholine salt	12.4	16.0	22.0	20.0	12.0	16.5



FIGS. 1–2.—Fig. 1 (*top*), reduction in stand of Dutch White clover in Kentucky bluegrass sod following treatment on March 15, 1946, with ammonium salt of 2,4-D at rate of 4 lb. per acre applied dry in mixture with sand. Area (⅛ acre) at center of photograph was treated; adjacent areas left untreated. Photographed July 9, 1946. Fig. 2 (*bottom*), eradication of narrow-leaved plantain by application on March 22, 1946, of 2,4-D applied dry in mixture with fertilizer. *Left*, plot which received fertilizer only; *center*, untreated guard strip; *right*, plot which received fertilizer containing 2,4-D applied at rate of 3 lb. per acre. In each instance complete fertilizer (10–6–4) was applied at rate of 600 lb. per acre. Photographed May 15, 1946.

either the acid or the salt forms of 2,4-D, caused the sod to develop a dark-green coloration as compared with that of adjacent areas which received fertilizer only (fig. 1). This deep-green color persisted until fall. On May 29, clippings were obtained on strips 18 inches wide and 25 feet long selected at random at five different locations in the treated areas (table 3).

The high percentage of weed clippings found in the control areas consisted mainly of narrow-leaved plantain, sheep sorrel, and white clover. The ammonium salt reduced the amount of weeds more effectively than did the other salts. Direct comparison between the salts and the acid cannot be made because of the difference in time and manner of application (the salts were applied in mixture with sand, the particles of which were more uniform in size than were those of the fertilizer). The acid and the sodium salt treatments were about equal in effectiveness, while the morpholine salt was relatively ineffective as a herbicide when applied in this manner.

Discussion

The use of a mixture of 2,4-D and complete fertilizer for weed control and fertilizing purposes would appear to have a number of advantages over other methods of treating grassy areas with 2,4-D. Except for the added cost of incorporating 2,4-D in the mixture, the treatment would cause no additional expense in ridding such areas of weeds. The herbicidal treatment would fit into the regular schedule of spring or fall fertilization recommended for lawn care, and the use of costly labor and equipment for applying a special 2,4-D spray treatment for weed-killing could therefore be avoided.

General recommendations for the use of fertilizer plus 2,4-D mixture cannot be made on the basis of the present limited experiments. Results of the present tests indicate, however, that the inclusion of a small amount of 2,4-D in lawn fertilizer mixtures would prove effective in destroying a large percentage of established weeds and would discourage reinfestation from weed seed present in the soil.

It has been shown in previous experiments (**1, 4, 6**), as well as in those reported herein, that many weedy plants are quite sensitive to 2,4-D absorbed through the roots. The effects of the chemical appear to be translocated rapidly throughout the entire plant within a short time after it is absorbed. This method of applying 2,4-D to the soil surface appears to offer promise in eradicating certain weeds that have large underground storage organs which frequently develop new shoots and reestablish the plant following spray treatment of the tops.

These experiments were conducted with freshly prepared mixtures of 2,4-D and fertilizer. Further experiments are in progress on the possible effect of the type of fertilizer used and its method of preparation and storage on the stability of 2,4-D contained in such mixtures.

Summary

1. Dry mixtures of 2,4-dichlorophenoxyacetic acid (2,4-D) and complete 10-6-4 fertilizer were applied to established weedy turf on November 6, 1945, and March 22, 1946, on plots 2 feet wide and 25 feet long. The fertilizer was used at the uniform rate of 600 lb. per acre and contained varying amounts of 2,4-D, namely, 0, 1.5, 3, 6, and 9 lb. per acre. The effects of the various treatments on the growth of turf and on the eradication of weeds were measured by sorting and weighing separately the clippings of weeds and of grass from the individual plots.

2. No significant difference, in comparison with the fertilized control, was found in the weight of grass clippings on April 4, 1946, from any 2,4-D fertilizer treatments that were applied on November 6, 1945. Such weeds as narrow-leaved plantain, black medic, and bur clover were eradicated almost 100% by the lowest amount (1.5 lb. per acre) of 2,4-D used in the fertilizer mixture. At the highest rates (6 and 9 lb. per acre) the green coloration of the grass was visibly darker than in the fertilized control plots until about June 1.

3. Application on March 22, 1946, of both 6 and 9 lb. per acre of 2,4-D in mixture with fertilizer caused visible injury to the turf so that the weight of grass clippings on May 6, 1946, was significantly lower than in the fertilized controls. However, in these experiments mixtures containing 1.5 and 3 lb. per acre of 2,4-D with fertilizer induced a highly significant increase in growth of the turf. Weed eradication was about equal to that resulting from treatment on November 6.

4. The bluegrass in all plots treated on November 6, 1945, was allowed to develop and to mature seed stalks, which were collected on June 6, 1946. The average dry weight of seed and chaff was 4.2, 17.0, 23.5, 22.1, 17.5, and 22.5 gm., respectively, for the control, fertilized control, and fertilizer plus 1.5, 3, 6, or 9 lb. of 2,4-D per acre. Owing to the absence of weeds, grass seed from the plots treated with 2,4-D was free of weed seeds.

5. Applications of the ammonium, sodium, and morpholine salts of 2,4-D mixed in dry sand were made on March 15, 1946, at the rate of 4 lb. per acre of each salt to individual $\frac{1}{6}$-acre plots of weedy turf. Samples of both weed and grass foliage from these plots on May 29, 1946, indicate that these salts of 2,4-D are effective in eradicating turf weeds when applied in dry form. The ammonium salt proved to be superior to the other salts in its herbicidal effects.

6. Although the present experiments are not sufficient to justify recommendations regarding the use of 2,4-D–fertilizer mixtures on turf, the lowest rate (1.5 lb. per acre) of 2,4-D that was used in combination with fertilizer caused almost 100% weed eradication. The chemical appears to be very effective when applied broadcast. It seems to be absorbed from the soil by the roots of sensitive weedy plants, and, once it has been taken up, the 2,4-D stimulus appears to be translocated rapidly throughout the entire plant.

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SOILS AND AGRICULTURAL ENGINEERING
BELTSVILLE, MARYLAND

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RESPONSE OF POTATO PLANTS TO SPRAY APPLICATIONS OF CERTAIN GROWTH-REGULATING SUBSTANCES[1]

ORA SMITH, M. A. BAEZA, AND J. H. ELLISON

Introduction

For years farmers have had heavy losses from potatoes sprouting excessively and shriveling in storages which could not be kept at temperatures of 40° F. or lower. Potato-chip manufacturers are constantly troubled with the production of dark-colored chips. This darkening is associated with high reducing-sugar content which develops at low storage temperatures. Higher storage temperatures would prevent accumulation of reducing sugars but would result in excessive sprout growth of tubers. Potatoes can now be treated in commercial quantities in storage with the methyl ester of α-naphthaleneacetic acid in liquid form (**8**) which prevents or greatly retards sprout growth at relatively high temperatures. The problem would be further simplified if some substance sprayed on the plants in the field would similarly retard growth of sprouts in storage and not reduce yields or quality of tubers. It was the purpose of this investigation to determine the effects of several compounds sprayed on potato plants on the mineral nutrient content of petioles, specific gravity and reducing-sugar content of tubers, yields of tubers, and effects on sprout retardation in subsequent storage.

Material and methods

Field tests were conducted on Lordstown silt loam soil of pH 5.0, using the Sebago variety, planted May 15, 1945, in rows 34 inches apart and with seed pieces at 11-inch intervals. All plots were fertilized alike at the time of planting with 2400 lb. to the acre of 5–10–10 fertilizer applied in equal depth bands through the planter.

The experiment included control plots and plots treated with four different growth-regulators applied in sprays: (*a*) sodium 2,4-dichlorophenoxyacetate at 10 p.p.m.; (*b*) methyl ester of α-naphthaleneacetic acid (NA) at 10,000 p.p.m. first application, 4000 p.p.m. thereafter; (*c*) indoleacetic acid (IA) at 150 p.p.m.; and (*d*) α-naphthaleneacetic acid (NA) at 150 p.p.m.

The spray solutions were made up in the following manner: The powder form of 70% sodium 2,4-dichlorophenoxyacetate directly soluble in water was used. Two parts of the methyl ester of NA plus 1 part of polyethylene glycol 400 were diluted with water to the desired strength and kept agitated during spraying. The concentration of the methyl ester of NA for the first application (10,000 p.p.m.) was so strong that marked injury to leaves resulted; hence, for all subsequent applications concentrations were reduced to 4000 p.p.m. Indoleacetic acid (IA) and α-naphthaleneacetic acid (NA) were each dissolved in 5 ml. of 95% ethyl alcohol and then diluted with water.

There were four dates of application: July 14 and 28 and August 11 and 25. One series of plots received applications on July 14 only, one series on July 14 and 28, one series on July 14 and 28 and August 11, and one series on all four dates. The sprays were applied through a knapsack sprayer at the following rates per acre: July 14, 55 gallons; July

[1] Paper no. 283, Department of Vegetable Crops, Cornell University, Ithaca, N.Y.

28, 82 gallons; August 11 and 28, 110 gallons. Plots were of single rows, 25 feet long with guard row between treatments and 5-foot alleys between replication blocks. All treatments were in quadruplicate.

Rapid chemical tests of petiole tissue were made on August 22 and September 8, using the methods of PEECH and ENGLISH (6). Plots were harvested and yield data were obtained on October 22, 1945. The specific gravity of the total tubers from each plot was obtained by differences in weight in air and in water. Samples from each plot were placed in storage at 40° F. and at 50° F. on November 15, 1945.

On February 1, 1946, the reducing-sugar content of tubers held at 40° F. was determined by a modified SHAFFER-SOMOGYI method (7).

On February 13, 1946, the number and weight of sprouts of the lots stored at 50° F. were obtained.

Results

GROWTH IN THE FIELD.—Two days after the first application plants of all plots sprayed with the methyl ester of NA showed severe upward rolling of the leaves, with some of the leaves severely injured. It appeared, however, that only those leaves receiving direct application of the chemical were injured.

SMITH *et al.* (9) found that the methyl ester of NA applied to the soil as a preemergence spray is an effective selective herbicide in potato fields. Although the 10,000 p.p.m. applications in the present experiment did not act as a complete herbicide to the potato plants, it was obvious that this was too high a concentration when the methyl ester of NA was sprayed on the plants; therefore, at the three subsequent applications concentrations were reduced to 4000 p.p.m. Two weeks after the first application plants treated with this substance had few flowers, while the untreated were flowering profusely. Most of the treated plants had somewhat recovered from the injury. Two weeks after the last application it was impossible to differentiate among those that had received one and those which had received more than one application. No effects were noticeable in the field on plants sprayed with the other growth substances.

RAPID CHEMICAL TESTS OF TISSUES.—Analyses of petiole samples taken between the third and fourth spray applications showed that there were no significant differences in nitrate nitrogen, calcium, or magnesium content of petioles owing to treatments or number of applications (table 1). The phosphorus content of petioles from plants sprayed with the methyl ester of NA was significantly higher than in all other treatments, and the potassium content of petioles from all spray treatments was significantly higher than in the untreated plants.

Results of analyses of petioles 2 weeks after the final applications had been made are shown in table 2. Petioles from plants of all treatments, except those sprayed with NA, were significantly higher in nitrate nitrogen than the untreated ones. At this time plants treated with IA and with the methyl ester of NA were significantly higher in phosphorus and potassium than the untreated ones. No significant differences among treatments were found in content of calcium or magnesium.

EFFECTS ON YIELDS.—Treatment with the methyl ester of NA significantly reduced the total yield and yield of U.S. no. 1 size potatoes (table 3). None of the other growth substances influenced yield significantly, and the number of spray

TABLE 1

EFFECT OF DIFFERENT GROWTH SUBSTANCES AND NUMBER OF APPLICATIONS ON CONTENT OF NUTRIENTS IN PETIOLES. AUGUST 22, 1945

TREATMENT	NUMBER OF APPLICATIONS																			
	Nitrate nitrogen (p.p.m.)				Phosphorus (p.p.m.)				Potassium (p.p.m.)				Calcium (p.p.m.)				Magnesium (p.p.m.)			
	1	2	3	Av.	1	2	3	Av.	1	2	3	Av.	1	2	3	Av.	1	2	3	Av.
								***				***								
Sodium 2,4-dichlorophenoxyacetate	800	608	880	763	45	45	55	48	8320	5230	7040	6880	600	600	500	580	35	25	25	28
Methyl ester of naphthaleneacetic acid	800	960	880	880	75	80	80	78	7200	10400	8000	8533	500	600	700	600	25	25	30	27
Indoleacetic acid	800	768	320	629	60	60	45	55	9600	6400	6400	7466	500	600	600	580	30	25	40	32
Naphthaleneacetic acid	640	560	360	587	50	45	50	48	6720	5600	7520	6613	600	600	700	590	25	25	30	27
Untreated*	688	688	688	688	50	50	50	50	4800	4800	4800	4800	600	600	600	600	30	30	30	30
Av.	746	717	666	.....	56	56	56	....	7328	6496	6752	.....	560	600	620	.....	29	26	31	....
	Not significant				L.S.D.—Treatment at .05 = 12 at .01 = 17 *** at .001 = 24				L.S.D.—Treatment at .05 = 1196 at .01 = 1661 ***at .001 = 2310				Not significant				Not significant			

* Samples from only two untreated plots taken; each figure represents average of same two plots.

TABLE 2

EFFECT OF DIFFERENT GROWTH SUBSTANCES AND NUMBER OF APPLICATIONS ON CONTENT OF NUTRIENTS IN PETIOLES. SEPTEMBER 8, 1945

Treatment	Number of applications																								
	Nitrate nitrogen (p.p.m.)					Phosphorus (p.p.m.)					Potassium (p.p.m.)					Calcium (p.p.m.)					Magnesium (p.p.m.)				
	1	2	3	4	Av.	1	2	3	4	Av.	1	2	3	4	Av.	1	2	3	4	Av.	1	2	3	4	Av.
					**					**					**										
Sodium 2,4-dichlorophenoxyacetate	450	300	600	450	450	30	50	65	100	61	7500	4950	7500	6000	6488	800	1000	800	800	850	7.5	17.5	7.5	7.5	10.0
Methyl ester of naphthaleneacetic acid	750	825	825	375	694	90	70	90	95	86	7500	8250	8250	6750	7688	700	800	700	900	775	5.0	7.5	7.5	5.0	6.2
Indoleacetic acid	450	600	300	600	488	75	80	70	95	80	7500	8250	8250	7500	7850	700	800	800	800	775	12.5	10.0	12.5	12.5	10.2
Naphthaleneacetic acid	300	300	300	525	356	70	65	80	95	78	6750	6000	5700	5400	5962	800	800	900	900	850	10.0	12.5	25.0	22.5	17.5
Untreated*	300	300	300	300	300	65	65	65	65	65	6750	6750	6750	6750	6750	800	800	800	800	800	7.5	7.5	7.5	7.5	7.5
Av.	450	465	465	450	....	66	66	74	90	....	7200	6840	7290	6480	.....	760	840	800	840	....	8.5	11.0	12.0	11.0	.....
	L.S.D.—Treatment at .05 = 144 ** at .01 = 197					L.S.D.—Treatment at .05 = 14 ** at .01 = 19 L.S.D.—Number of applications at .05 = 12 at .01 = 17					L.S.D.—Treatment at .05 = 1058 ** at .01 = 1447					Not significant					Not significant				

* Samples from only two untreated plots taken; each figure represents average of same two plots.

TABLE 3

EFFECT OF DIFFERENT GROWTH SUBSTANCES AND NUMBER OF APPLICATIONS ON TOTAL YIELD AND YIELD OF U.S. NO. 1 SIZE POTATOES. SEBAGO VARIETY. 1945

Treatment	Bushels per acre									
	Total					U.S. no. 1 size				
	1 application	2 applications	3 applications	4 applications	Av.	1 application	2 applications	3 applications	4 applications	Av.
Sodium 2,4-dichlorophenoxyacetate	308	300	324	306	309	268	259	295	262	271
Methyl ester of naphthaleneacetic acid	214	170	143	162	172***	159	108	86	94	112***
Indoleacetic acid	288	348	318	329	320	248	304	266	288	276
Naphthaleneacetic acid	332	321	326	337	328	283	277	287	296	286
Untreated	299	334	327	310	317	266	270	293	283	278
Av.	288	294	287	289	.....	245	244	245	245	.....
	L.S.D.—Treatment (total) at .05 = 29.3 bus. at .01 = 38.9 bus. *** at .001 = 50.6 bus. Number of applications—no significant difference					L.S.D.—Treatment (U.S. no. 1 size) at .05 = 28.6 bus. at .01 = 38.0 bus. *** at .001 = 49.4 bus. Number of applications—no significant difference				

TABLE 4

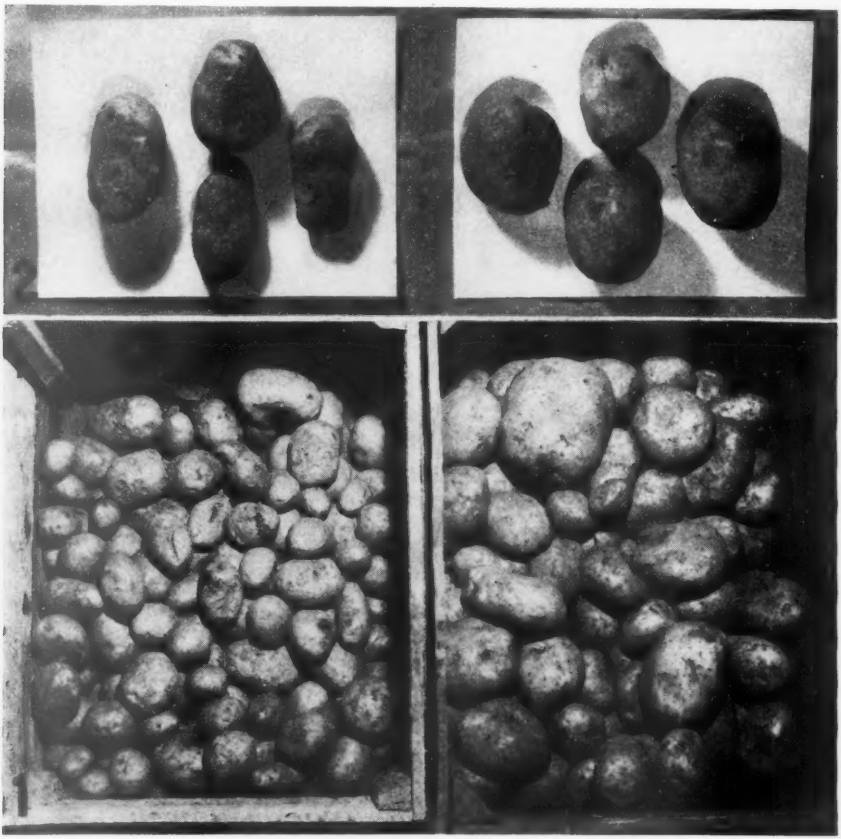
DEGREE OF INJURY EXHIBITED BY TUBERS FROM PLANTS SPRAYED WITH METHYL ESTER OF NA*

Treatment	Total number tubers	Percentage of area injured					Number with cracks
		No injury	Very little injury	25% injury	50% injury	More than 50% injury	
Untreated	138	100.0	.....	.....	.....	.....	2
Sprayed once	129	74.4	14.7	2.3	5.4	3.1	1
Sprayed twice	96	18.8	21.9	26.0	14.6	18.8	10
Sprayed three times	127	18.1	29.9	23.6	8.7	19.7	9
Sprayed four times	113	23.9	14.2	15.0	15.9	31.0	6

* Data refer to injured side of potato only.

applications had no significant effect on yield of tubers. Treatment with the methyl ester of NA significantly decreased the percentage of U.S. no. 1 size tubers as compared with the other treatments.

TUBER INJURY.—Typical injury to tubers and the size of tubers from plants which had been sprayed with the methyl ester of NA are shown in figures 1 and 2. The "pitted-scab-like" injury which was so prevalent occurred on only one side of the tuber and appeared to be confined to the underside of the tubers as they were attached to the plant. Often the opposite or upper side contained a prominent growth crack. The injury extended several millimeters into the tuber, affecting the periderm and a few cell layers of the cortex. There were marked differences in amount and severity of injury, increasing with number of spray



FIGS. 1–2.—Fig. 1, each crate contains total yield from one plot. *Left*, tubers from plants sprayed four times with methyl ester of α-naphthaleneacetic acid; note characteristic injury resembling potato scab, growth cracks, and small size of tubers. *Right*, tubers from unsprayed plants. Fig. 2, tubers on left from plants sprayed four times with methyl ester of α-naphthaleneacetic acid, tubers on right from unsprayed plants; note differences in size, shape, and surface injury.

TABLE 5

EFFECT OF DIFFERENT GROWTH SUBSTANCES AND NUMBER OF APPLICATIONS ON SPECIFIC GRAVITY OF POTATOES. SEBAGO VARIETY

TREATMENT	SPECIFIC GRAVITY				
	1 application	2 applications	3 applications	4 applications	Av.
Sodium 2,4-dichlorophenoxyacetate	1.063	1.061	1.062	1.061	1.062
Methyl ester of naphthaleneacetic acid	1.059	1.052	1.054	1.046	1.053***
Indoleacetic acid	1.061	1.062	1.060	1.062	1.062
Naphthaleneacetic acid	1.063	1.061	1.063	1.062	1.063
Untreated	1.064	1.062	1.061	1.062	1.062
Av.	1.062**	1.060	1.060	1.059	

L.S.D.—Treatment
*** at .001 = .003460
L.S.D.—Number of applications
** at .01 = .0023

TABLE 6

EFFECT OF DIFFERENT GROWTH SUBSTANCES AND NUMBER OF APPLICATIONS ON REDUCING-SUGAR CONTENT DEVELOPED BY POTATOES STORED FOR 11 WEEKS AT 40° F.

TREATMENT	REDUCING SUGAR (MG./CC. JUICE)				
	1 application	2 applications	3 applications	4 applications	Av.
Sodium 2,4-dichlorophenoxyacetate	6.16	6.77	5.45	5.68	6.01
Methyl ester of naphthaleneacetic acid	5.67	5.75	4.94	5.18	5.39
Indoleacetic acid	4.60	6.61	6.82	5.69	5.93
Naphthaleneacetic acid	6.20	6.07	4.70	6.95	5.98
Untreated	5.60	5.59	5.32	6.18	5.67
Av.	5.60	6.16	5.45	5.94	N.S.

No significant differences

applications (table 4). A similar type of injury has been noted by ENNIS *et al.* (2) as a result of spraying potato plants with ammonium 2,4,5-trichlorophenoxyacetate.

SPECIFIC GRAVITY OF TUBERS.—A high positive correlation exists between specific gravity, starch content, and dry weight of potatoes and the texture or degree of mealiness. Hence, the mealiness of tubers can be measured by the specific gravity method, and they subsequently are available for further chemical analysis or other determinations or for planting.

Spray treatment and number of spray applications resulted in statistically significant differences in specific gravity of the tubers (table 5). Treatment with the methyl ester of NA lowered the specific gravity markedly; specific gravity in all other treatments was the same as in the untreated lots.

REDUCING-SUGAR CONTENT OF TUBERS.—*After 11 weeks' storage at 40° F.*, reducing-sugar analyses were made of tubers from each treatment. The kind of treatment and the number of applications had no significant effect on the reducing-sugar content developed in storage (table 6).

SPROUTING OF TUBERS IN STORAGE.—At the end of 3 months' storage at 50° F. samples of twenty tubers were chosen from each lot, and data were obtained on number and weight of sprouts. Those stored at 40° F. showed no visible sprouts at that time. Table 7 shows that all spray treatments had significantly decreased the number of sprouts per tuber; very marked reduction resulted from applications of methyl ester of NA (figs. 3–4). There was no significant difference in number of sprouts per tuber as related to number of spray applications of any of the chemicals. The relationship was

TABLE 7

EFFECT OF DIFFERENT GROWTH SUBSTANCES AND NUMBER OF APPLICATIONS ON SPROUTING OF POTATOES STORED FOR 3 MONTHS AT 50° F. SEBAGO VARIETY. 1945

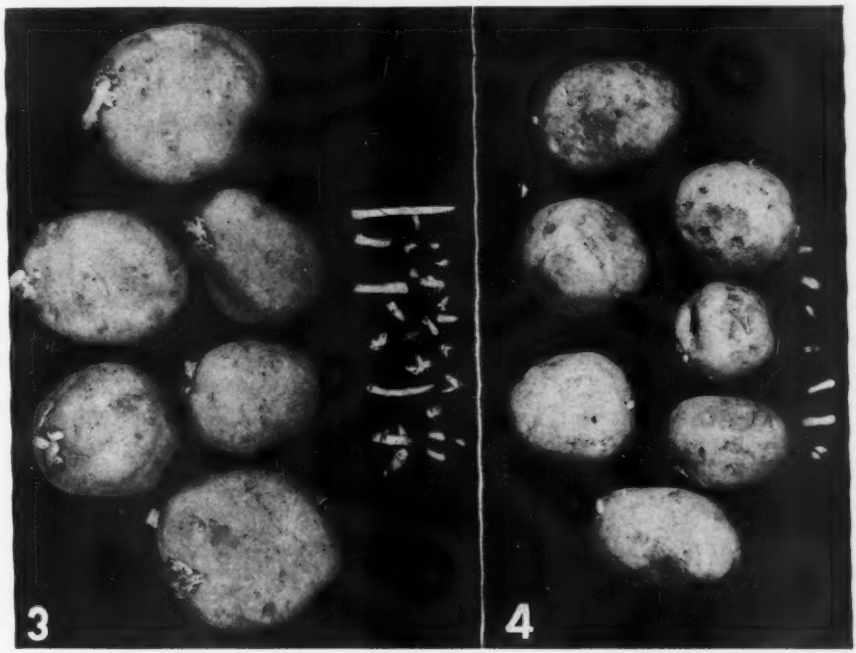
TREATMENT	NUMBER OF SPROUTS PER TUBER					WEIGHT PER SPROUT (MG.)				
	1 application	2 applications	3 applications	4 applications	Av.	1 application	2 applications	3 applications	4 applications	Av.
Sodium 2,4-dichlorophenoxyacetate	4.5	3.9	3.8	4.6	4.2*	150	180	170	140	160**
Methyl ester of naphthaleneacetic acid	3.2	1.2	1.8	0.91	1.7***	130	120	140	160	140**
Indoleacetic acid	4.7	4.2	3.9	3.9	4.2*	200	180	190	150	180
Naphthaleneacetic acid	5.2	3.4	4.6	4.0	4.3*	160	120	190	170	160**
Untreated	5.1	6.3	5.1	4.9	5.4	220	160	150	220	190
Av.	4.5	3.8	3.8	3.4	.....	170	150	170	170	.....
	Number of applications—no significant difference L.S.D.—Treatment * at .05 = 0.9752 at .01 = 1.29 *** at .001 = 1.68					Number of applications—no significant difference L.S.D.—Treatment at .05 = 22 ** at .01 = 29				

much the same for weight per sprout, an indication of size of sprout, as it was with number of sprouts.

Discussion

Spray applications of the methyl ester of naphthaleneacetic acid and of indoleacetic acid resulted in an increase in the petiole content of nitrate nitrogen, phosphorus, and potash, compared with those from unsprayed plants. Applications of sodium 2,4-dichlorophenoxyacetate resulted in increased petiole content of nitrate nitrogen at the second sampling and of potassium at the first date of sampling. STUART (11) found that large amounts of nitrogen and carbohydrates moved from other portions of bean plants to the portion which had been treated with indoleacetic acid. Naphthaleneacetic acid applications increased the potassium content of petioles at the first period of sampling. Application of none of the growth substances resulted in a significant decrease, compared with untreated plants, in petiole content of any of the five mineral nutrients.

There is some evidence that applications of certain growth substances to plants result in mobilization of certain nutrients and metabolized products in the area of application. In the present experiment possibly application of growth substances to the leaves resulted in an accumulation of certain nutrients in the petioles.



FIGS. 3–4.—Fig. 3, six tubers from control plants (unsprayed), showing amount of sprout growth after 3 months' storage at 50° F.; removed sprouts from six similar tubers. Fig. 4, seven tubers from plants sprayed three times during growing season with methyl ester of α-naphthaleneacetic acid, showing sprout growth and typical "pitted-scab-like" surface injury; removed sprouts from seven similar tubers.

HAMNER (4) found that relative percentage contents of calcium, phosphorus, and nitrogen in the bean plant were increased by the presence of naphthaleneacetamide in the nutrient solution. This may have been apparent rather than real in relation to other components, for SWARTZ (12) published as follows: "A significant decrease in total dry weight of the Marigold plants supplied with naphthaleneacetic acid (0.01 mg. per liter) indicates a retarding effect of the acid on growth." This agrees with the results of our experiment, in which yields of tubers were significantly reduced by the use of the methyl ester of NA.

There does seem to be some disagreement among workers using growth-regulators. For instance, LAUDE (5), working with the Red Kidney bean, *Phaseolus vulgaris*, found that, under certain levels of nutrition, increases as great as 12.5% in total plant dry weight were obtained by the use of low concentrations of indoleacetic acid in the nutrient solution.

The only treatment which resulted in any significant difference in yield compared with untreated plants was the application of the methyl ester of NA. The decrease in yield may have been due to injury to the leaves, particularly from the early applications, or to some alteration in the metabolism of the plant which resulted in accumulation of certain constituents in the leaves and stems rather than in the tubers.

That the methyl ester of NA sprayed on leaves and stems is translocated to the tubers is indicated by the fact that most tubers produced by such plants had injury similar to pitted scab on their under surfaces and to the fact that sprout growth was retarded in tubers from sprayed plants as it is on tubers treated in storage with the same substance. The dry weight of tubers from plants sprayed with the methyl ester of NA, as shown by specific gravity measurements, was significantly lower than that of tubers from any other treatment. SMITH and NASH (10) have shown that specific gravity of tubers may be influenced by many factors, such as variety, time of planting and of harvest, fertilizer treatments, light intensity, temperature during the latter portion of the growing season, maturity, and soil moisture. Immature tubers are of lower specific gravity than mature tubers. None of the above listed factors can be used to explain logically the lower specific gravity of tubers from plants receiving applications of the methyl ester of NA.

No significant differences in reducing-sugar content of tubers held 11 weeks at 40° F. could be found among any of the treatments. DENNY *et al.* (1) found no definite effect on the reducing-sugar content of the juice of tubers treated with the methyl ester of NA.

Spray applications of all the growth substances to plants in the field resulted in a significant decrease in amount of sprout growth of tubers subsequently stored at 50° F. The methyl ester of NA was much more effective in retarding sprout growth than any of the other growth substances. GUTHRIE (3) obtained inhibition of sprout growth of potatoes by treating whole or cut tubers with the potassium salt of indoleacetic acid, the potassium salt of naphthaleneacetic acid, and the methyl ester of NA.

Summary

1. Spray applications of the methyl ester of α-naphthaleneacetic acid (NA), sodium 2,4-dichlorophenoxyacetate, α-naphthaleneacetic acid, and indoleacetic acid were made to field-grown Irish potatoes at several stages of growth.

2. Applications of the methyl ester of NA at 10,000 p.p.m. at the rate of 55 gallons of mixture to the acre resulted in severe upward rolling of the leaves, with some of the leaves being injured severely. Rapid chemical tests of petiole tissues of plants sprayed with the methyl ester of NA showed a higher content of nitrate nitrogen, phosphorus, and potassium than in those of untreated plants. Yields were reduced, most tubers exhibited a "pitted-scab-like" injury, specific gravity of tubers was lowered, and sprout growth of tubers was inhibited as a result of spray applications of the methyl ester of NA. Applications of sodium 2,4-dichlorophenoxyacetate (10 p.p.m.), naphthaleneacetic acid (150 p.p.m.), and indoleacetic acid (150 p.p.m.), at rates varying from 55 to 110 gallons to the acre, had no visible effects on vine growth and did not affect yields of tubers, specific gravity of tubers, or reducing-sugar content of tubers after 11 weeks at 40° F. storage, compared with the untreated plants.

3. Petioles of plants sprayed with indoleacetic acid were higher in nitrate nitrogen, phosphorus, and potassium than those of unsprayed plants. Those sprayed with sodium 2,4-dichlorophenoxyacetate were higher in nitrate nitrogen and potassium; those sprayed with naphthaleneacetic acid were higher in potassium.

4. Tubers from all the sprayed treatments, after 3 months' storage at 50° F., had significantly less sprout growth than tubers from untreated plants.

DEPARTMENT OF VEGETABLE CROPS
CORNELL UNIVERSITY

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GROWTH OF FIELD CROPS IN SOIL TREATED WITH CHEMICAL GROWTH-REGULATORS

D. L. TAYLOR

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 581

Introduction

Reports of studies at Camp Detrick, Frederick, Maryland (**7**), emphasized the possibilities of the use of chemical growth-regulators as soil or spray treatments for controlling weeds in connection with general agricultural programs. Isopropyl-N-phenyl carbamate was found to inhibit markedly the development of seedlings of several common grasses, while most dicotyledonous species appeared to be little affected by such treatments. The possibility of using 2,4-dichlorophenoxyacetic acid to inhibit selectively the growth of weeds seemed likely considering the tolerances of the acid by a number of crops, especially grasses.

More recent studies (**2, 5, 6**) have dealt with treatment of soils, inactivation of seeds, seedling development, and the degree of persistence of activity of treatment and of viability of weed seeds in the soil. In some instances the soil was pretreated or treatments could be so construed.

It has been suggested that treatment of the soil before planting crops could afford a desirable variation of herbicidal technique (**2, 6**). Much remains to be defined, however, regarding the quantity, intensity, and timing of such treatment in relation to specific crops, the environment, and residual effects of treatments. Such effects could be reflected in the soil response, about which very little is yet described, in the development of seeds and seedlings, and in the later growth of plants on treated soil.

Tests involving treatment of the soil were conducted in an effort to obtain further information about weed control, persistence of effects of treatments, and the growth of field crops on soil pretreated with 2,4-dichlorophenoxyacetic acid (2,4-D) or isopropyl-N-phenyl carbamate (IPPC).

Material and methods

These field tests were conducted from April through September, 1946, at Lake Geneva, Wisconsin. Two areas of light to medium, loamy top soil were used. One soil had relatively low fertility, contained marl (crumbly clay and $CaCO_3$), and was alkaline, pH 7.8–7.9. The second soil, a medium-light silt loam, was of moderate fertility and had a pH of 6.9–7.0. During previous seasons both soils had been cultivated.

The 2,4-D was a white powder obtained from Dow Chemical Company. Isopropyl-N-phenyl carbamate (IPPC), which was secured from Sherwin-Williams Company, was a gray-white, granular solid (m.-p. 87°–89° C.).

The test crops, selected because of ease of cultivation and economic importance, were Red Kidney bean, Henry spring wheat, white mustard, Golden Bantam sweet corn, and Silver King (Wisconsin no. 7) white dent corn. Kidney bean and mustard are highly sensitive to 2,4-D and apparently insensitive to IPPC. Wheat and corn are sensitive to IPPC and relatively insensitive to 2,4-D, wheat being less so than corn.

Investigation

Test 1

Procedure.—On April 25 all weeds were removed from the test areas and 2,4-D or IPPC was applied at the rate of 0, 2, or 4 lb. per acre. Ten and five-tenths or 21 gm. of the chemicals, passed through a sixteen-mesh sieve, were thoroughly mixed into 50 lb. of lake sand, and passed through an eight-mesh sieve. Each mixture was broadcast evenly over test areas of 12 × 42 feet to supply the chemicals at the rate of 2 or 4 lb. per acre, respectively. All areas were raked over lightly once to mix the dressings into the top $\frac{1}{2}$ inch of soil.

On each planting date all weeds which had appeared following treatment were removed from the parts of treated areas to be planted. Weeds were allowed to develop on the remainder of the treated areas, until these were required for plantings, in order to observe the degree of weed control on the unplanted ground.

Except in the plot treated with IPPC, the initial planting was made on April 25. Nine other plantings were made in all areas on the 7th, 14th, 21st, 28th, 31st, 35th, 38th, 45th, and 52d days (June 16) after treatment. At each planting twenty-five seeds each of corn, bean, and wheat were planted approximately 1.5 inches deep in separate rows, 20 inches apart.

Observations were made on the amount of, and time required for, emergence of shoots, the number and general condition of seedlings 30 days after planting, and the number and size of mature plants. Some corn plants were also harvested when they were at mid- to late-vegetative stage of development.

Weather.—Between April 25 and September 25, 16.3 inches of rainfall were recorded at the center of the experimental area. More than two-thirds of it fell during the first 2 months (table 1). Precipitation was one and a half and two times normal in May and June, respectively, but during the remainder of the period it was only 40% of the 44-year normal. Throughout the summer the extremes and means of daily temperatures were in general 1°–2° F. less than normal.[1]

Rate of seedling emergence.—When plantings were made within a week after the soil was treated with 2,4-D, the time required for emergence of seedlings from half of the corn or wheat seeds was 1 or 2 days, respectively, more than for the controls; for beans, 12–16 days (fig. 1). The 2-lb. treatment affected wheat and bean seedling emergence in successive plantings for 5–6 weeks. This persistence effect was irregular and less than 5 weeks in duration on emergence of corn seedlings for both 2- and 4-lb. treatments but lasted about 7 weeks for wheat and beans in soil pretreated with 4 lb. of 2,4-D per acre. Thus the duration of inhibitory residual effects was somewhat proportional to initial rate of treatment.

The IPPC treatment had slight and irregular, if any, effect on the emergence of bean, but markedly decreased and slowed the emergence of the cereals (fig. 1). Again corn appeared less sensitive than wheat. Not until seed was planted 4 weeks after treatment did 50% emergence of wheat occur, and then emergence of the much-stunted seedlings was delayed 7–10 days in comparison with the controls. The number of emerging corn seedlings, which were delayed for 8–20 days depending on the interval between soil

[1] The author is indebted to the Yerkes Observatory of the University of Chicago, Williams Bay, Wis., and to Miss Mary Calvert for information concerning average rainfall and temperature and for data on temperatures for 1946.

treatment and planting, was less than half that of the control if plantings followed within 2 weeks after treatment.

SEEDLING NUMBER AND CONDITION.—Observations were made on the general condition and numbers of seedlings 30 days old in relation to time of emergence of seedlings and survival of plants to maturity. Beans, when planted up to 5 weeks following application to the soil plantings made within 4 weeks after treatment with 2,4-D. The tops of control plants appeared to be two or more times as large as those for plantings made within 2 or 6 weeks following application of 2 or 4 lb., respectively, of 2,4-D per acre.

Development of root systems was curtailed and abnormal when beans were planted within 2 weeks after treatment

TABLE 1

SUMMARY OF DATA ON RAINFALL AND TEMPERATURE AT LAKE GENEVA, WISCONSIN, DURING MAY THROUGH SEPTEMBER, 1946

Rainfall and temperature	May	June	July	August	September
	Rainfall (in inches)				
Recorded, 1946	4.06	7.35	1.91	1.74	1.19
Difference from normal	+ 1.67	+ 3.69	− 1.46	− 2.04	− 2.64
	Temperature (in °F.)				
Mean recorded, 1946	55.5	66.0	72.6	68.2	64.0
Difference from normal	− 2.0	− 1.6	− 0.3	− 2.7	+ 0.5
Mean maximum recorded, 1946	66.5	76.5	83.6	79.3	76.0
Difference from normal	− 2.1	− 1.9	− 0.5	− 2.5	+ 2.2
Mean minimum recorded, 1946	44.5	55.4	61.6	57.0	52.0
Difference from normal	− 2.0	− 1.6	− 0.3	− 2.7	+ 0.5

of 2,4-D at the 2-lb. rate, had shoots slightly smaller than the controls and showed distinct symptoms of 2,4-D effect on the leaves—especially attenuated, abnormally shaped primary leaves. Leaf symptoms which developed when seed was planted 45 days after the 4-lb. treatment were soon outgrown.

No growth-regulator treatment of soil appeared to induce an increase in the size of seedling shoots of any planting of beans. Inhibition of shoot was distinctly visible on seedlings 30 days old from of soil with 2,4-D. In most instances only curved hypocotyls emerged from the soil. Normal-appearing roots often developed at subsurface cotyledonary nodes of such plants, and a few shoots emerged very tardily from the soil. Inhibited misshapen primary root systems of some such plants were completely elevated by subsequent straightening of the hypocotyls.

In plantings of beans made within 2 weeks after treatment the populations of seedlings were smaller, in proportion to

the rate of application of 2,4-D, than in untreated soil. Seedlings, 30 days old from plantings made within 3 weeks after the soil was treated with 2,4-D, were less able to survive to maturity than control seedlings (fig. 2).

Pretreatment of soil with 4 lb. of 2,4-D per acre reduced the size of populations of wheat seedlings distinctly less than of beans (fig. 2). The decreases of wheat were less in soil treated with 2 lb. of the acid per acre. The percentage of survival of wheat seedlings to maturity from plantings made within 4 or 5 weeks after soil treatment was lowered slightly by both rates of application.

The sizes and subsequent survival of populations of corn seedlings indicated that, of the crops tested, corn was least inhibited by the 2,4-D treatments. When plantings were made 4 or 5 weeks after pretreatment of soil with either rate of application, shoots appeared normal in size after 30 days of growth. In plantings made more than 5 weeks after treatment, seedlings 30 days old seemed normal and somewhat larger than in untreated soil.

Treatment of soil with IPPC did not appear to influence markedly the survival of bean seedlings (fig. 2). With wheat, however, the sizes of populations and the inability of many seedlings to survive if plantings were made within 5 weeks after treatment reflected the distinct inhibitory effect of IPPC on this crop. With corn, by the end of 30 days, populations were reduced 75% or more if planted within 2 weeks after treatment (fig. 2), but seedlings developed from three-fourths of the corn seed planted 3 weeks after treatment. With later plantings, the treatment appeared to have no consistent effect on corn seedling populations or their survival. Subsequent survival of 30-day-old seedlings was greater for corn than for wheat when plantings were made within 3 weeks after treatment.

WEED RESPONSES.—Growth of weeds was distinctly reduced by pretreatment of soil with 2,4-D. IPPC did not influence the growth of dicotyledonous weeds and did not appear distinctly effective against yellow bristle grass, *Setaria lutescens* (Weigel) F. T. Hubb., or crab grass, *Digitaria sanguinalis* (L.) Scop.—the major weed grasses present.

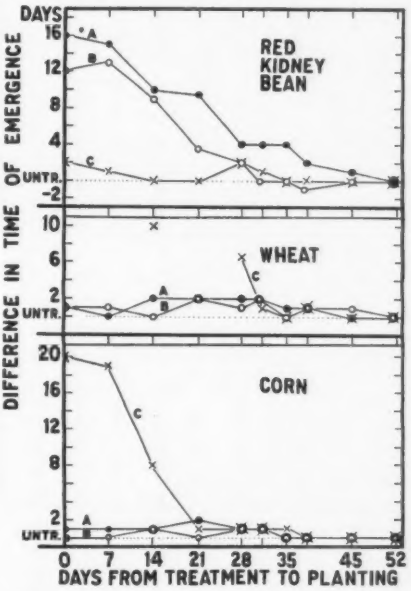

FIG. 1.—Differences in time required for emergence of bean, wheat, or corn seedlings from half of seeds planted in untreated soil and in soil pretreated with (*A*) 4 lb. of 2,4-D per acre, (*B*) 2 lb. of 2,4-D per acre, or (*C*) 4 lb. of IPPC per acre. Wheat did not emerge when plantings were made immediately, 7 days, or 21 days after soil was treated with IPPC. Numbers of days required for emergence of seedlings from successive plantings in untreated soil were: bean, 18, 13, 11, 11, 11, 9, 9, 8, 9, 10; wheat, 12, 10, 7, 6, 6, 5, 7, 5, 5, 7; and corn, 11, 8, 7, 8, 6, 6, 8, 6, 7, 9, respectively.

All the planted areas were again weeded during the third week in June and the last week in July because weeds were ex-

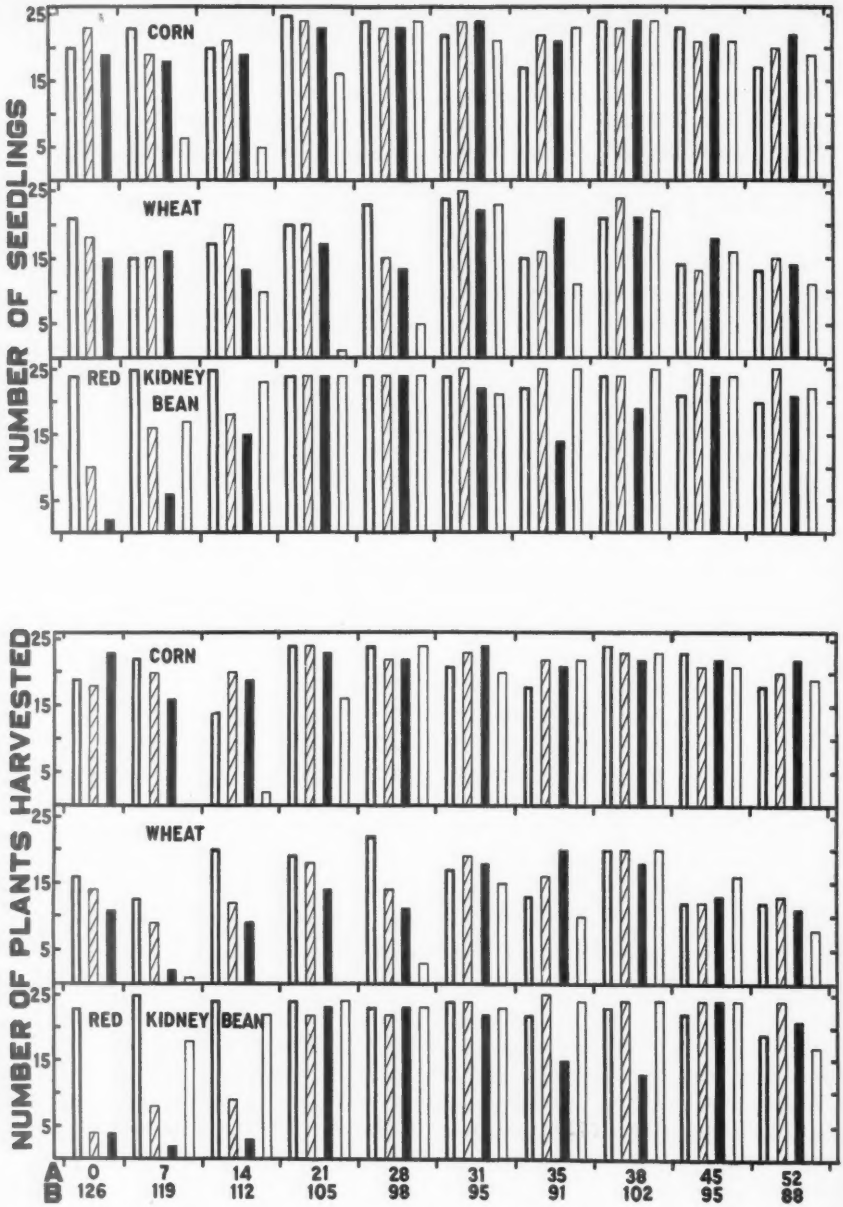

FIG. 2.—Numbers of bean, wheat, and corn plants present at 30 days (*upper*) or harvested (*lower*) when twenty-five seeds of each were sown at intervals up to 52 days following treatment of soil with 2,4-D or IPPC. Four bars which represent each planting denote soil treatments: *left bar*, untreated soil; *hatched bar*, 2 lb. of 2,4-D per acre; *black bar*, 4 lb. of 2,4-D per acre; *right bar*, 4 lb. of IPPC per acre. Numbers along line *A* indicate time in days between treatment of soil and planting. Age in days of plants harvested from different plantings is shown on line *B*.

cessive in the untreated and in the IPPC-pretreated areas.

WEIGHT OF VEGETATIVE TOPS OF CORN.—During June and July, when conditions prevailed which were favorable for the growth of corn, it became apparent that plants in soil pretreated with 2,4-D were exceeding others in size. On August 1, when 98–46 days old, one-half of the plants from each planting were harvested. The silks were then dry on corn planted within 2 weeks after soil was treated and were functional on plants from the succeeding four plantings. Corn planted 38 or 45 days after soil treatment was tasseled and coming into tassel, respectively. In the last planting the tassels had not emerged.

In the first six plantings (made within 31 days after soil treatment) the fresh weight of tops of corn grown in soil treated with 2 lb. of 2,4-D was greater than the controls by 6–58% (table 2). In such soil the weights of tops of later plantings were less regularly related to treatment conditions and approximately were equal to the controls or exceeded them by up to 50%. When maximum drought conditions prevailed during the period when major growth normally would have occurred (plantings 6 and 7), the weights of shoots were most nearly equal for plants grown in treated and untreated soil.

Corn planted 2 weeks or more after treatment of soil with 4 lb. of 2,4-D per acre had stems which were 12–45% heavier than plants grown in untreated soil. As the length of the period between soil treatment and planting date increased, the weights of the shoots of such treated plants tended to exceed by greater amounts those grown in soil treated with 2 lb. per acre. It did not appear that 2,4-D had otherwise altered the general developmental sequence of corn.

Pretreatment with IPPC resulted in shoots of smaller weight as compared with controls when plantings were made up to about 5 weeks after soil treatment. The appearance of tassels and silks appeared to be delayed for 1–2 weeks. Later plantings were not so influenced by the soil treatment.

POPULATION AND WEIGHT OF TOPS OF MATURE PLANTS.—Mature crops at ages of 91 days, or more, which had been planted within 5 weeks, or less, after the time the soil was treated were counted and harvested on August 29. In order to allow them to mature, the later plantings were not harvested until September 12, when they were 102–88 days old. At the time of harvest all beans had finished flowering and pods were full, wheat was past the "milk-dough" stage, and the ears of corn were filled out.

Some older leaves of wheat plants more than 90 days old had dried, and plants 15 weeks old were dry. During the growth period it appeared that flowering of beans growing in soil treated with 2,4-D had been delayed 10–14 days. Bean plants grown in soil pretreated with growth-regulators, especially with IPPC, remained green longer than controls.

Pretreatment of soil with 2,4-D reduced numbers of beans which survived to maturity compared with controls if plantings were made up to 2 weeks after treatment (fig. 2). This response occurred in wheat planted within 4 weeks of the time of treatment. The numbers in mature corn populations were limited only slightly, if at all, when plantings followed treatment of the soil with 2,4-D by 1 week (fig. 2).

When soil was pretreated with IPPC, numbers of bean plants were not limited compared with controls if plantings were made more than 1 week after treatment.

Numbers of corn or wheat plants were reduced if plantings followed this treatment within 3 or 4–5 weeks, respectively.

The fresh weight of tops of mature bean plants in soil pretreated with 2 lb. of 2,4-D per acre was lower than the controls for the first five plantings (table 3) and for the first six plantings in soil treated at the 4-lb. rate. Tops grown in soil treated with IPPC were heavier than the controls when plantings were made within 5 weeks after treatment. Bean plants grown in soil treated with 2,4-D were heavier and those in IPPC-treated soil were lighter than the controls when planted more than 5 weeks after the soil was treated (table 3).

Vegetative stems of corn and mature

TABLE 2

AVERAGE FRESH WEIGHTS OF TOPS OF VEGETATIVE AND MATURE CORN PLANTED AT INTERVALS UP TO 52 DAYS AFTER SOIL WAS TREATED WITH 2,4-DICHLOROPHENOXYACETIC ACID (2,4-D) OR ISOPROPYL-N-PHENYL CARBAMATE (IPPC)

Date of planting	Length of time (days)		Average weight of tops per plant			
	Treatment to planting	Growth period	Untreated soil (gm.)	Percentage difference* from tops in untreated soil when soil was pretreated with—		
				2,4-D		IPPC
				2†	4†	4†
	Vegetative					
April 25	0	98	632	+58	− 6	Not planted
May 2	7	91	613	+32	− 4	−27
May 9	14	84	649	+45	+21	−32
May 16	21	77	630	+ 6	+25	−42
May 23	28	70	605	+32	+27	−26
May 26	31	67	671	+ 8	+12	−27
May 30	35	63	731	−13	+19	−24
June 2	38	60	541	+23	+29	− 2
June 9	45	53	585	+ 2	+15	− 3
June 16	52	46	307	+50	+45	+15
	Mature					
April 25	0	126	644	+50	+17	Not planted
May 2	7	119	765	+27	+59	No harvest
May 9	14	112	835	+18	+12	−28
May 16	21	105	722	+50	+35	−45
May 23	28	98	881	+34	+ 2	−40
May 26	31	95	824	− 4	− 6	−16
May 30	35	91	743	− 8	− 5	−12
June 2	38	102	681	+ 3	+18	− 6
June 9	45	95	652	+15	− 3	−19
June 16	52	88	802	+ 5	− 2	−12

* Relative average weight of tops of corn grown in treated soils is calculated in relation to value of 100% assigned to average weight of top per plant of same age grown in untreated soil.

† Equivalent pounds per acre.

tops of wheat were lighter than the controls when planted less than 40 days after the soil was pretreated with IPPC. Mature tops of corn were lighter than the controls for all plantings in the IPPC-treated soil.

The tops of wheat plants were approximately equal to the controls in weight for plantings made 1 or 2 weeks following treatment of the soil with 2 lb. of 2,4-D per acre (table 3). Unavoidable loss of some dry mature parts of plants on treated soil may have accentuated the differences between their weights and those of the controls planted 2–6 weeks after treatment. Tops of wheat planted within 6 weeks after the soil was treated with 4 lb. of 2,4-D per acre were up to 75% lighter than those of controls, depending on the pretreatment period.

The tops of mature corn plants from plantings made within 3–4 weeks after application of 2,4-D were consistently heavier than controls by 12–59% (table 2). Stems from later plantings in treated and untreated soil were about equal in size, whereas the vegetative tops of such plants in soil pretreated at the 4-lb. rate had been heavier than controls. Vegetative shoots produced from plantings made within a week after application of this treatment had been about equal to control plants in weight.

TABLE 3

AVERAGE FINAL FRESH WEIGHTS OF TOPS OF BEAN AND WHEAT PLANTS GROWN FOR PERIODS OF 88–126 DAYS AFTER SEEDS WERE SOWN AT INTERVALS UP TO 52 DAYS FOLLOWING PRETREATMENT OF SOIL WITH 2,4-D OR IPPC

Date of planting	Length of time (days)		Average weight of tops per plant							
	Treatment to planting	Growth period	Untreated soil (gm.)		Percentage difference* from tops in untreated soil when soil was pretreated with—					
					2,4-D				IPPC	
					2†		4†		4†	
			Bean	Wheat	Bean	Wheat	Bean	Wheat	Bean	Wheat
April 25	0	126	183	6.8	−26	−19	−15	−59	Not planted	Not planted
May 2	7	119	202	4.5	−33	− 2	−74	−78	+41	No stand
May 9	14	112	193	5.0	−54	+20	−80	−62	+22	No stand
May 16	21	105	172	8.3	−21	−35	−69	−41	+ 8	No stand
May 23	28	98	133	11.6	−11	−33	−24	−28	+44	−38
May 26	31	95	122	9.3	+19	−14	−32	−42	+33	+20
May 30	35	91	132	10.9	+33	−26	+20	−44	+22	−16
June 2	38	102	127	13.5	+15	−22	0	−42	−13	−21
June 9	45	95	175	6.5	+24	− 1	− 9	0	−13	+17
June 16	52	88	207	7.9	+23	−14	+42	+25	−20	− 9

* Relative average weight of tops of plants grown in treated soils is calculated in relation to value of 100% assigned to average weight of top per plant of same age grown in untreated soil.

† Equivalent pounds per acre.

TEST 2

Late in May, when shoots of corn plants growing in soil pretreated with 2,4-D began to appear larger than the controls, the question of the magnitude of the effect of weed competition arose.

A second test was initiated to attempt to determine whether the apparent greater growth of corn in treated soil was due to the competition between corn and weeds in the untreated area or to an actual stimulation of growth resulting directly from treatment of the soil.

PROCEDURE.—The methods were in most ways similar to those used in test 1. All weeds were removed from an area of silt-loam soil which did not contain marl and which had a pH of 6.9–7.0. This area was divided into three parts, each 8 × 25 feet, by shallow trenches.

On June 2 two parts were treated with 2,4-D at rates of 1 or 3 lb. per acre. The third part was left untreated. Plantings were made in one-third of each area 20, 30, or 40 days after treatment. Immediately before each planting all weeds were removed from all treated areas. After the planting on the 20th day, weeds were not removed from the areas planted at that time, but the remaining parts of the treated areas were kept free of weeds throughout the test period.

At each planting 64 grains of sweet corn and 30 white mustard seeds were sown in each area. Such mustard seedlings as developed were removed 20 days after planting by which time their response had indicated any residual inhibitory activity of the soil treatments. The amount and time of emergence of seedlings and the growth of weeds in the areas treated 20 days before planting were observed. Half of the corn from each planting was harvested when 48 (late vegetative) or 72 days old (fully reproductive).

RESULTS.—By 10 days after mustard seeds were sown in soil pretreated for 20 days with 1 or 3 lb. of 2,4-D per acre, emergence was 13% or 7%, respectively, compared with more than 75% emergence in untreated soil. Seedlings in treated soil were much stunted. No further emergence from treated soil occurred. Less than 50% of the mustard seed produced seedlings, except when sown 40 days after treatment. Their emergence was delayed for 1–2 days, and strong symptoms of effect of treatment with 3 lb. of 2,4-D were evident in this last planting.

The numbers of corn seedlings which emerged from the 20-day planting in pretreated soils were equal to those in the controls, but emergence was delayed for a day. Similar delay resulted for corn planted 30 days after soil had been treated with 3 lb. of 2,4-D per acre. In the 30- and 40-day plantings 15–35% more seedlings emerged during the period of drought from pretreated than from untreated soil. Survival of seedlings to maturity did not appear to be influenced by the treatments.

Suppression of the number and size of weeds, which principally were purslane, *Portulaca oleracea* L., chickweed, *Alsine media* L., and yellow bristle grass, in areas treated with 1 or 3 lb. of 2,4-D per acre was 85% or 95%, respectively, compared with weeds in untreated soil. To some extent reduction was proportional to rate of treatment, although all areas pretreated with 2,4-D were remarkably free from weeds.

Three weeks after the first planting, corn plants in soil treated with 3 lb. of 2,4-D per acre appeared as large as the controls, and those in soil treated with 1 lb. appeared larger than controls. Plants in untreated soil appeared to grow more slowly than those in treated soil as the growth period progressed. There was no indication that 2,4-D treatments influenced the time of appearance of tassels or silks.

Corn of the 20- and 30-day plantings was in tassel, just showing silks, and the

40-day planting was only in tassel when 48-day-old plants of each of these lots were harvested. When plants 72 days old were harvested, they were green and ears were edible. In the plantings made 30 days after treatment many ears of control plants were less well filled than on plants grown in treated soil.

Fresh weights of vegetative and reproductive tops (table 4, fig. 3) grown in treated soil were distinctly greater than in the controls. When pretreatment was with 1 lb. per acre, the tops of 48-day-old plants from the 20-day planting were heavier than those in soil treated with 3 lbs. per acre. This difference was half as great when the plants were 72 days old. For other plantings and treatments this relationship was reversed.

When plantings were made more than 20 days after treatment, differences in the intensity of treatment did not result in differences in the weights of tops with the exception of the 72-day plants from the 40-day planting. In the latter case treatment with 3 lb. of 2,4-D per acre resulted in larger plants. It is evident that stimulation in the growth of corn resulted from pretreating the soil with 2,4-D, whether weeds were allowed to grow and compete or were removed by cultivation.

TABLE 4

TOTAL YIELDS AND AVERAGE WEIGHTS OF TOPS OF CORN GROWN IN UNTREATED SOIL AND IN SOIL PRETREATED WITH 2,4-D 20–40 DAYS BEFORE PLANTING

Planting		Growth-regulator treatment (lb./acre)	Total harvest			Average fresh weight and % difference in average weight of tops per plant harvested at—			
				Weight and % difference in weight		48 days		72 days	
Time after treatment (days)	Management		No. of plants	Kg.	%	Gm.	%	Gm.	%
20	Not weeded	0	52	7.65	.....	132	.....	162	.....
	Not weeded	1	54	13.64	+78	211	+60	294	+81
	Not weeded	3	51	11.48	+50	180	+36	272	+68
30	Weeded	0	48	10.60	.....	153	.....	288	.....
	Weeded	1	46	15.73	+48	258	+69	425	+48
	Weeded	3	55	18.49	+74	250	+63	425	+48
40	Weeded	0	34	4.74	.....	107	.....	172	.....
	Weeded	1	38	5.87	+24	146	+36	163	− 5
	Weeded	3	47	8.05	+70	145	+35	197	+15

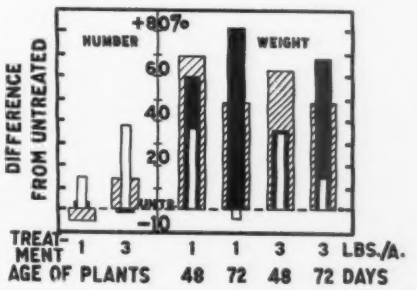

FIG. 3.—Relative differences from controls (*Untr.*) in numbers of plants harvested and in average fresh weights of 48- or 72-day-old tops from corn planted 20 days (*solid bars*), 30 days (*hatched bars*), or 40 days (*white bars*) after soil was treated with 1 or 3 lb. of 2,4-D per acre.

Discussion

In these field tests a desirable inhibition of weed growth resulted, and re-

sponses of test crops indicated that residual activity of IPPC or 2,4-D persisted in soil approximately 5 or 7 weeks, respectively. Some inhibition of the growth of kidney beans or wheat occurred in plantings made up to 5 or 6 weeks, respectively, after the soil was treated with 2,4-D. Bean shoots were slightly heavier than controls if plantings were made $4\frac{1}{2}$ weeks, or more, after the soil was treated with 2,4-D or within 5 weeks after soil treatment with IPPC. The rate and amount of growth of corn was distinctly stimulated when soil was pretreated with 2,4-D.

Dicotyledonous weeds were almost completely eliminated throughout the growing season on neutral and alkaline, medium-light, field soil which was treated at the start of the season with 1–4 lb. of 2,4-D per acre. IPPC did not influence the growth of such weeds. The growth of yellow bristle grass and crab grass was slightly greater in untreated soil than in soil treated with 2,4-D or IPPC during periods which lasted not more than 3–4 weeks after treatment.

In general, the development of beans and mustard was more inhibited than that of wheat and corn in proportion to intensity of 2,4-D treatment (treatment rate × time interval between treatment of soil and planting); IPPC had more effect on the cereals. The responses of test crops in soil treated with 4 lb. of 2,4-D per acre were, for the most part, parallel to but delayed for about 2 weeks compared with those for the 2-lb. treatment.

TABLE 5

LENGTH OF TIME (WEEKS) REQUIRED BETWEEN TREATMENT OF SOIL AND SOWING OF SEED IN ORDER THAT PLANTS IN SOIL TREATED WITH 2,4-D AND IN UNTREATED SOIL WERE SIMILAR CONCERNING VARIOUS CRITERIA

Criterion	Treatment (lb./acre)	Crop tested		
		Bean	Wheat	Corn
Time required for emergence of seedlings	2	$4\frac{1}{2}$	5	5
	4	7	6	3–5
Number of seedlings emerged during 30 days after planting	2	3	1	0
	4	4–6	$4\frac{1}{2}$–5	3–4
Visible symptoms of deleterious effects on tops	2	$5\frac{1}{2}$	$4\frac{1}{2}$	4–$4\frac{1}{2}$
	4	$7\frac{1}{2}$	$6\frac{1}{2}$–$7\frac{1}{2}$	5
Number of mature plants	2	3–4	$4\frac{1}{2}$	1–2
	4	4–6	6	3–4
Survival of seedlings after 30 days	2	3	3	1
	4	3	4–$4\frac{1}{2}$	2
Weight of mature tops	2	$4\frac{1}{2}$	5–6	0
	4	5	5–6	1–2

Table 5 summarizes the duration of inhibitory responses in test 1. The combined effects of strong inhibition of root systems of bean, abnormal shoot growth of which was appreciable, and drought condition during the period when major growth normally occurred seem the most plausible explanation for the small sizes of populations (fig. 2) and the low weight of mature plants (table 3) from plantings made 5 or 6 weeks after treatment of soil

with 4 lb. of 2,4-D per acre. Data on other crops for these plantings are not contradictory to this explanation. The relatively large weights of mature shoots of wheat for plantings made 1–2 weeks after treatment with 2,4-D may in part have been due to unavoidable loss of parts of control plants which were completely dry before harvest. Relatively heavy shoots from the last planting in soil treated with 4 lb. per acre may in part have resulted from the effect of 2,4-D treatment in slightly prolonging the green condition of plants.

Responses likely resulted from the influence of the calcium salt of 2,4-D formed by reaction with marl ($CaCO_3$) in the soil which was low in organic matter. It should be recalled in considering residual activity that 11.5 or 8.5 inches of rain fell during 6–8 weeks immediately after treatment of soil in tests 1 or 2, respectively. Also, the temperature during the first 2 weeks of test 1 was unfavorable for growth of kidney beans and corn.

On the basis of the criteria described, some residual inhibitory activity of 2,4-D lasted in the soil for about 50 days compared with 95% survival of soybeans which DEROSE (1) reported for plantings made 90 days after 2,4-D was applied at the rate of 50 lb. per acre on silt-loam soil. In greenhouse tests MITCHELL and MARTH (6) found normal emergence of barley but "reduced" emergence of mustard in a soil low in organic matter which had been treated with 4 or 14 mg. of 2,4-D per pound and then stored dry for 1 or 6 months, respectively.

Table 6 summarizes the duration of inhibitory effects of soil treatment with IPPC. There was little consistent effect of IPPC treatment on later plantings of corn or wheat. The green condition of wheat plants was slightly prolonged.

It appeared that temporary and inhibitory effects of IPPC activity persisted about 5 weeks in the soil compared with 7 weeks for 2,4-D when applications were made at the rate of 4 lb. per acre. This relation corresponds with that found in greenhouse tests with non-alkaline silt loam and in field tests where applications were made at the rate of 50 lb. per acre (1).

Dry leaves were almost lacking at harvest time on bean plants, 8–44% heavier than controls, for plantings made within 5 weeks after soil was pretreated with IPPC. The period during which this response was evident corresponds well with the period and intensity of residual activity indicated by other criteria. Prolongation of the green condition of tops of beans for plantings made $4\frac{1}{2}$ weeks or more after treatment of soil with 2,4-D must have contributed to the relatively heavier tops of these plants. However, such plants were visibly slightly larger and their lower stems thicker than those of control plants. Rather distinct apparent increases over the controls in the size of vegetative plants have been observed for kidney beans planted in

TABLE 6

LENGTH OF TIME (WEEKS) REQUIRED BETWEEN TREATMENT OF SOIL WITH 4 LB. OF IPPC PER ACRE AND SOWING OF SEED IN ORDER THAT PLANTS IN TREATED AND IN UNTREATED SOIL WERE SIMILAR CONCERNING VARIOUS CRITERIA

Criterion	Crop tested		
	Bean	Wheat	Corn
Time required for emergence of seedlings	1–2	5–6	4–5
Number of seedlings emerged during 30 days after planting	2	5	4–5
Visible symptoms of deleterious effects on shoots	1–2	$5\frac{1}{2}$	$4\frac{1}{2}$
Number of mature plants	2	5	4
Survival of seedlings after 30 days	0	4–5	3
Weight of mature tops	0	5	5–6

soil which had been in moist warm storage for 1–2 months following application of 2,4-D (4).

The most striking response was the distinctly greater growth of corn in soil pretreated with 2,4-D than in untreated soil (tables 2–4). Compared with controls, tops were distinctly (13%) lighter only for one planting and were distinctly heavier (12–60%) for a majority of the twenty plantings made in treated soil in test 1. Stimulation was most consistent when plantings were made within 1 month after treatment and was greatest for plantings which followed within 2 weeks after soil was treated with 2 lb. of 2,4-D per acre. The vegetative stems of corn for early sowings in soil treated with 4 lb. per acre weighed less than those in soil treated at the 2-lb. rate but were practically equal to controls. In soil treated at the 4-lb. rate, vegetative corn plants were consistently heavier (12–45%) than controls in later plantings. HANKS (3) found an apparent stimulation of root elongation when the corn bio-assay technique was used to measure residual activity in leachates obtained from different soils which had been held in moist storage for 2–6 weeks after treatment with the acid or its calcium salt. The response was most consistent for sandy loam stored for 2 weeks or alkaline silt loam stored for 6 weeks.

The length of the period of vegetative growth and the total numbers of corn plants available for harvest did not appear distinctly influenced by 2,4-D treatment. Survival of plants which were most subjected to drought condition (40-day planting in test 2) appeared to be slightly greater in soil pretreated with 2,4-D than in untreated soil (table 4, fig. 3).

Mature stems of corn grown in treated soil were 12–59% heavier than controls for plantings made within 1 month after treatment in test 1 and 15–60% heavier in weed-free soil for plantings made 30 or 40 days after treatment in test 2. Weed competition in untreated soil was undoubtedly an influencing factor in test 1 and for the 20-day plantings in test 2. In the latter case the ground was not weeded, and lush weed growth occurred in untreated soil, while treated areas remained relatively free of weeds.

On the bases of weight of vegetative or mature tops, growth of corn in treated soil was greater or more rapid than the controls when plantings were made 30 days after treatment; when made 40 days following treatment, the difference was lessened (table 4). Control plants grew faster, or more, during the latter part of the growth period than plants in treated soil when plantings were made more than 4 weeks after treatment in test 1 or when plantings were made 30 days or more after treatment in test 2.

It seemed that more rapid growth occurred in soil treated with 2,4-D but that final weights of plants for later plantings were more nearly equal in untreated and treated soil. This is clearly evident for the 40-day planting in test 2. Apparent stimulation of growth was consistent and permanent in appreciable amount when corn was planted 30 days or less after treatment of the soil with 2,4-D.

Changes in the amount of crop responses which indicated deleterious or inhibitory influence of soil treatments with 2,4-D seem quite compatible with a progressive decrease of residual toxic activity during 7 weeks. This occurrence in combination with a progressive decrease in a residual stimulating influence could account, at least in part, for the relatively greater growth during the latter part of the growth period by control plants for later plantings and likewise for rela-

tively greater growth of corn for earlier plantings in soil treated with 4 lb. of 2,4-D per acre.

Viewed as a whole, the results of these tests strongly indicate that with proper management of treatment a combination of weed control with desirable stimulation of crop growth might be possible.

Summary

1. In two field tests 2,4-dichlorophenoxyacetic acid (2,4-D) and isopropyl-N-phenyl carbamate (IPPC) were applied as top dressings in dry form at rates from 1 to 4 lb. per acre on neutral or alkaline loam soils at the start of the growing season. Successive plantings of Red Kidney beans, mustard, wheat, field corn, and sweet corn were made at intervals up to 52 days after the time of treatment of the soil.

2. Desirable control of weeds during the growing season resulted from treatment with 2,4-D, while IPPC did not influence dicotyledonous weeds and had only slight temporary inhibitory effect on weedy grasses.

3. Activity of the 4-lb. treatment of these growth-regulators, which was evaluated on test crops by means of the rate and amount of their emergence, number and condition of 30-day-old seedlings, survival of seedlings to maturity, and the amount of growth of tops, appeared to persist in the soil 7 weeks for 2,4-D and 5 weeks for IPPC. The growth of corn indicated that residual activity of the acid lasted longer than 7 weeks.

4. Growth-regulator treatments slightly prolonged the green condition of kidney beans; IPPC similarly influenced wheat.

5. Weights of shoots of mature bean or wheat plants grown in untreated soil and in soil treated with 4 lb. of 2,4-D per acre were equal when plantings were made 5–6 weeks after treatment.

6. The weights of shoots of kidney beans were generally slightly heavier than controls if plantings were made more than 4 weeks after treatment of the soil with 2,4-D.

7. Shoots of corn grew more rapidly and were heavier in soil pretreated with 2,4-D than in untreated soil. This stimulation was considered in relation to weed competition and residual activity of treatment.

Department of Botany
University of Chicago

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SPECTROPHOTOMETRIC METHOD FOR DETERMINATION OF 2,4-DICHLOROPHENOXYACETIC ACID

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 582

ROBERT S. BANDURSKI

The use of 2,4-dichlorophenoxyacetic acid (2,4-D) as a differential herbicide applied directly to soil has stimulated investigation of the factors involved in its retention in that medium. Measurement of the persistence of the herbicide in stored soil has been accomplished by seedling emergence tests (3), and its concentration in the leachates of treated soils has been determined by HANKS (1) using the bio-assay method of SWANSON (5).

A spectrophotometric method for the determination of 2,4-D was developed in connection with a study of its retention by various soil components and synthetic ion-exchangers placed in contact with aqueous solutions of the acid. The method may be applied to the quantitative determination of 2,4-D in aqueous or ether solutions and in extracts of sand, clay, peat, and soil, provided that the concentration of other compounds which absorb light in the ultraviolet region does not become prohibitive.

SYNTHESIS AND PURIFICATION OF 2,4-D

Since commercial grades of 2,4-D may contain small amounts of compounds having chlorine atoms substituted at places other than the 2,4 positions, it was considered desirable to prepare this reagent. The method of synthesis, though not the purification procedure, is essentially that of POKORNY (4). Equimolar concentrations of 2,4-dichlorophenol (Eastman Kodak–white label), monochloroacetic acid (Merck's), and an excess of sodium hydroxide were placed in a small quantity of water and heated to near-dryness on a steam bath. The residue was washed with several portions of 95% ethanol and then dropped into a solution of 5% hydrochloric acid. The hydrochloric acid was decanted, and the crystals of 2,4-D were washed repeatedly with water. They were then dissolved in a minimal amount of 80% ethanol, the solution filtered, and the acid recrystallized by slow evaporation of 75% of the solvent. The large pure white crystals were then dried at 60° C. *in vacuo*. The melting-point of the product was 132°–134° C., which is not in good agreement with the figure of 138° C. reported by POKORNY. Earlier attempts to purify 2,4-D of technical grade by repeated salt-acid cycles and crystallization always resulted in a product having a melting-point of 132°–134° C. In spectrophotometric analyses the position of the maxima and the value of the extinction coefficients for purified technical 2,4-D and for that prepared by the above procedure were the same, within the limits of experimental error.

PHOTOELECTRIC SPECTROPHOTOMETRIC ANALYSIS

The absorption spectra of 2,4-D in aqueous and ether solutions were determined with a Beckman Quartz Spectrophotometer, model DU, using a hydrogen discharge tube as the light source and silica sample cells. Diethyl ether was chosen as one of the two solvents tested because 2,4-D can be quantitatively ex-

tracted from aqueous solutions of low pH by shaking with ether, thus providing a simple means of concentration of soil extracts.

Figure 1 presents absorption data for 2,4-D in aqueous and ether solutions. The numerical value of the extinction coefficient, k, is plotted on a logarithmic scale along the ordinate, and the wavelength, in angstrom units, along the abscissa. Beer's law is used in the form

$$k = \frac{1}{cx} \log_{10} \frac{I_0}{I_x},$$

where k is the extinction coefficient; c is the concentration in milligrams per milliliter; x is the thickness of the solution layer in centimeters; I_0 is the intensity of radiant energy transmitted by the solvent-filled cell; and I_x is the intensity

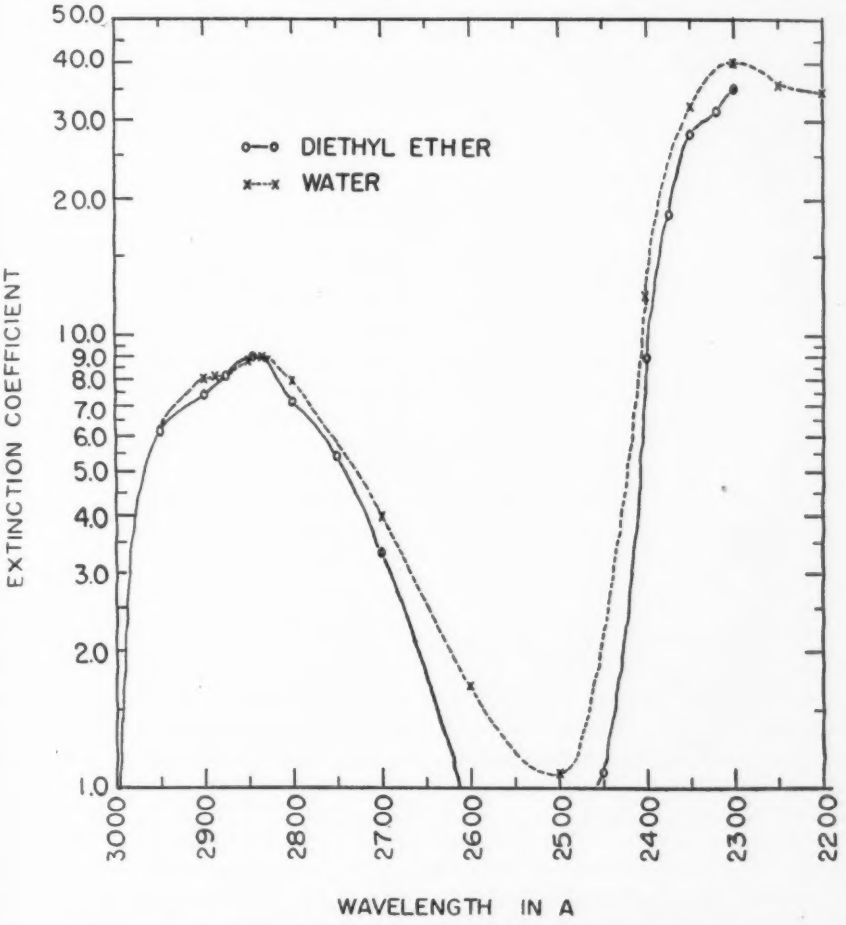

FIG. 1.—Absorption spectra of 2,4-dichlorophenoxyacetic acid in water and ether solutions

of radiant energy transmitted by the solution-filled cell.

Beer's law was found to hold within 5% limits over a concentration range of 6–250 γ/ml. The values of k for the acid in water, as measured at 2835 A and at 2300 A on a solution containing 25 γ/ml., were found to be independent of reaction over the range of pH 2.2–8.0; it is thus probable that Beer's law will hold for much greater dilutions. The absorption data are summarized in table 1.

TABLE 1

Solvent	Maxima (A)	Spectral region isolated, band width (A)	Extinction coefficient
Water....	2835	13	9.0
	2300	12	40.6
Ether.....	2845	17	9.0

TABLE 2

Wave-length (A)	Cell length (cm.)	Concentration (γ/ml.)	Computed concentration for maximum accuracy (γ/ml.)
2835.....	1.0	20–300	48.0
	5.0	3–50	9.6
	10.0	2–30	4.8
2300.....	1.0	5–70	11.0
	5.0	1–14	2.1
	10.0	0.5–7.0	1.1

Data are given in table 2 for the concentrations of 2,4-D which can be measured at the regions of maximum absorption with a minimum density of 0.2 and a maximum density of 2.8.

The computation of the optimum density for spectrophotometric work is discussed by LOOFBOUROW (2) following the method of TWYMAN and LOTHIAN (6). It is advisable to use a combination of concentration and cell length such that the values of $\log_{10}(I_0/I_x)$ fall within the range of 0.4–2.0 (7). If it is desired to work with solutions of very low densities, a calibration curve should be constructed.

Discussion

Though specific directions for measurement of 2,4-D in aqueous solutions containing other substances which absorb in the ultraviolet region cannot be given, owing to the wide variations in quantity and quality of the interfering substances, certain general recommendations may be made. In the case of extracts from clay, sand, and dilute aqueous extracts of peat, measurement of the concentration of the acid may frequently be made directly on the aqueous solution, after clearing of suspended matter. A blank solution, extracted under identical conditions with respect to pH, salt concentration, contact time, and temperature from a sample of the same medium not containing the acid, must be used. The reaction at which the solution density is determined can vary over the range of pH 2.2–8.0, although in strongly acid or alkaline solution the extinction coefficient varies, owing presumably to hydration effects.

If the concentration of substances which absorb in the ultraviolet region is too great to permit density determinations directly on the aqueous soil extract, recourse may be had to the following procedure. An aliquot of the solution is acidified (pH 3–4) and is extracted three times with a volume of diethyl ether equal to the volume of the aliquot. The volume of the ether may then be reduced, provided the volume of the ether extract of the blank is also reduced to the same point and made to the same volume.

Summary

1. A simple and accurate spectrophotometric method for the determination of 2,4-dichlorophenoxyacetic acid in aqueous and ether solutions is described.

2. The extinction coefficients for the acid in water at 2835 A and at 2300 A, and in ether at 2845 A, have been determined.

3. Certain chemical and instrumental precautions essential to quantitative results are discussed.

The author takes pleasure in acknowledging the aid of Dr. ROBERT A. WHITMORE, who first located the maxima in ether at approximately 2840 A and who gave many helpful suggestions during the course of the work.

DEPARTMENT OF BOTANY
UNIVERSITY OF CHICAGO

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ON THE LIFE-HISTORY OF VANILLA PLANIFOLIA

B. G. L. SWAMY

Vanilla planifolia Andr., a native of Central America, is grown in South India as a horticultural plant. Material for the present study was obtained from plants growing in a private garden. Serial microtome sections were stained with Heidenhain's iron-alum haematoxylin following customary methods.

Investigation

MICROSPOROGENESIS.—The wall of the microsporangium shows an epidermis (figs. 1–4), a fibrous endothecium (figs. 3–5), one, or sometimes two, middle layers, and one or two layers of tapetal cells which are very prominent in early stages. Only the endothecium persists in the mature anther (fig. 5).

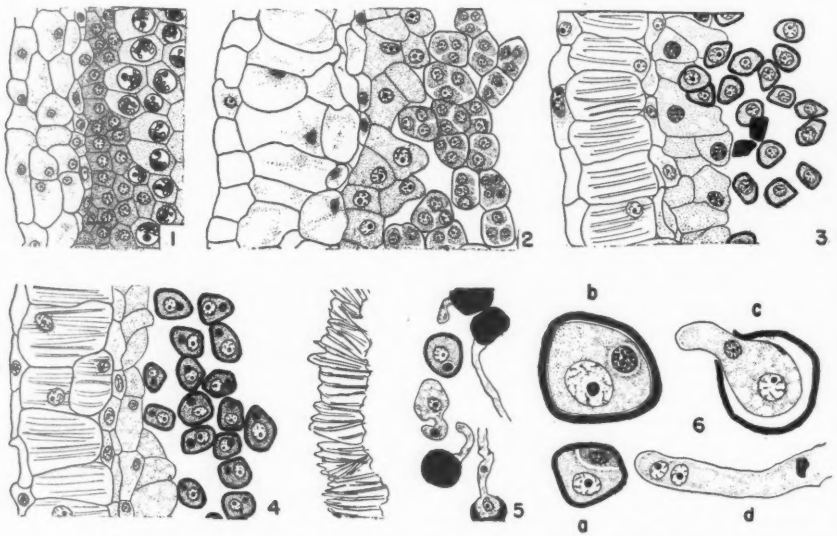
Just before the meiotic divisions, the polygonal microspore mother cells (fig. 1) begin to round up. The two divisions take place in rapid succession and are followed by inwardly directed furrows which result in the formation of the microspore tetrads (fig. 2). Unlike the condition in several other orchids, the microspores round up, develop a thick wall, and lie free in the anther sac (fig. 3).

The first division of the microspore cuts off a lens-shaped generative cell (fig. 6*a*), whose exact position in relation to the tetrad could not be determined,

owing to the very early separation of the microspores. After a time the generative cell, with its very small and densely staining nucleus, is seen to lie inside the plasma of the tube cell (figs. 5, 6*b*).

The pollen is usually shed at the two-celled stage, but in many anthers observed the pollen grains had already germinated and put forth long pollen tubes (fig. 5). It is always the generative

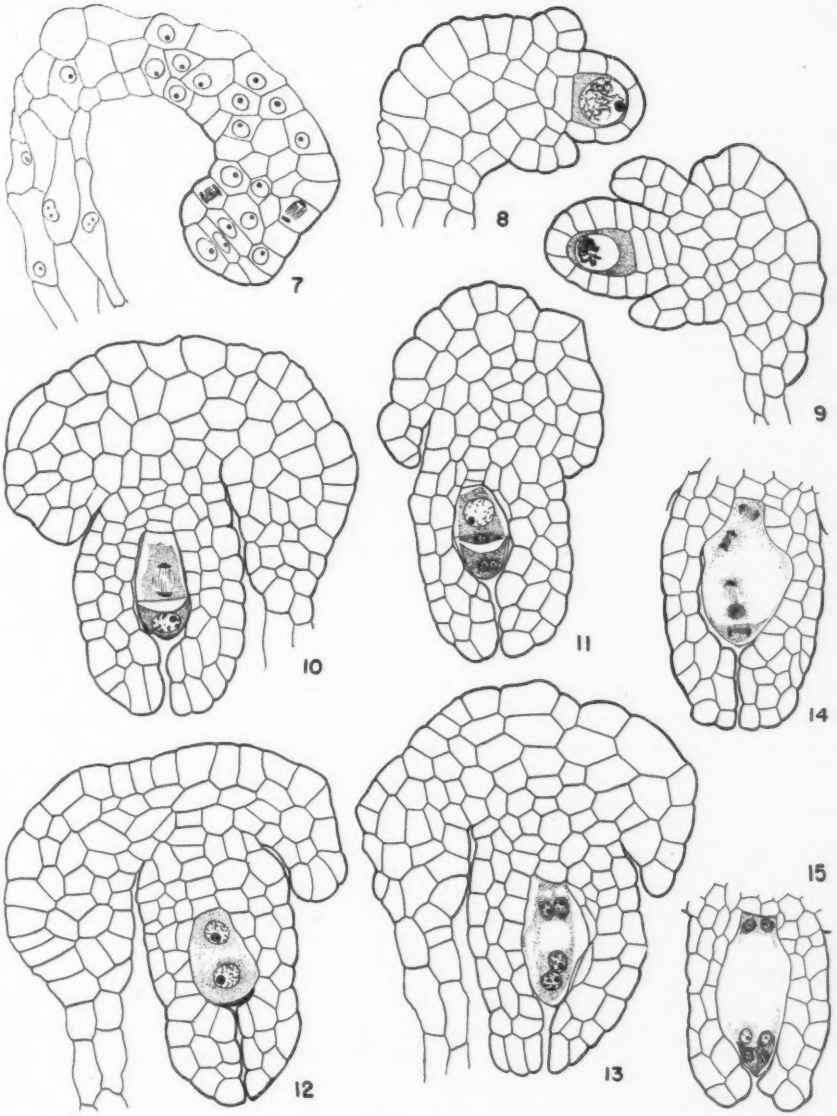
OVULE.—Differentiation of the ovules from the placental ridges starts only after pollination. In artificially pollinated flowers the archesporial cells become evident in the ovary 15–20 days after pollination. The placental ridges, which appear to be somewhat bilobed in a transverse section of the ovary, initiate divisions in the superficial cell layers and give rise to a number of papil-



FIGS. 1–6.—Fig. 1, part of young microsporangium showing wall layers, tapetum, and sporogenous tissue. ×225. Fig. 2, same at microspore tetrad stage. ×225. Fig. 3, uninucleate microspores in anther loculus; note fibrous endothecium. ×225. Fig. 4, two-celled pollen grains. ×225. Fig. 5, germination of pollen grains within anther. ×225. Fig. 6*a*, generative and tube cells in pollen grain soon after division of microspore. ×900. Fig. 6*b*, pollen grain at time of shedding. ×900. Fig. 6*c*, germination of pollen grain. ×900. Fig. 6*d*, tip of pollen tube showing degenerating tube nucleus and two male nuclei. ×900.

cell which moves into the pollen tube first, whatever may be its position in the pollen grain (figs. 5, 6*c*). During later stages, also, the degenerating tube nucleus can be seen behind the two sperm nuclei in the pollen tube (fig. 6*d*). Germination of pollen grains *in situ*, a curious feature in this plant, is connected with a tendency toward self-pollination, a subject which has been dealt with in detail elsewhere (4).

lae, each of which is three to four cells thick (figs. 7, 8). This is an important deviation from the great majority of orchids in which similar papillate outgrowths from the placenta occur at a corresponding stage but consist only of a unicellular filamentous row of five to six nucellar cells capped by the epidermis. It is also worth noting that in *Vanilla* the number of nucellar cells undergoes a further increase in the region below the



FIGS. 7–15.—Fig. 7, nucellar papilla before differentiation of archesporial cell. ×225. Fig. 8, archesporial cell. ×225. Fig. 9, megaspore mother cell and origin of integuments. ×225. Fig. 10, dyad cells; chalazal in second meiotic division. ×450. Fig. 11, row of three cells; two megaspores and micropylar dyad cell. ×450. Fig. 12, two-nucleate embryo sac. ×450. Fig. 13, four-nucleate embryo sac. ×450. Fig. 14, division of all four nuclei (an exceptional case). ×450. Fig. 15, mature six-nucleate embryo sac. ×450.

archesporial cell, including the area at the base of the two integuments (figs. 9–13).

The inner integument differentiates more or less simultaneously with the archesporium (fig. 8) and is two- to three-layered (figs. 10, 11). The outer integument, which appears when the megaspore mother cell is undergoing reduction divisions, is also made up of three to four layers of cells and may be even five- to six-layered at the base (figs. 10, 35, 38). This is interesting, for in other orchids it consists of only one layer, or rarely two layers, of cells. In later stages the outer integument completely envelops the inner but is separated from the latter by a broad air space (figs. 35, 38).

MEGASPOROGENESIS AND FERTILIZATION.—The archesporial cell enlarges in size and functions directly as the megaspore mother cell (figs. 8, 9). The first meiotic division takes place in the usual manner. The micropylar dyad cell is always smaller and more darkly staining than the chalazal one (fig. 10) and only rarely undergoes further division. The chalazal dyad cell completes the second meiotic division but here also the micropylar daughter cell is smaller and more darkly staining than the other. Thus there originates a row of three cells, consisting of a uni- or binucleate dyad cell and two megaspores (fig. 11). Only the chalazal megaspore develops further. By the time of the meiotic divisions the nucellar epidermis has degenerated so that the developing embryo sac is in immediate contact with the inner integument (figs. 10, 11, 12).

The two-nucleate embryo sac (fig. 12) gives rise to the four-nucleate one. At first all four nuclei are of approximately the same size and appearance (fig. 13). The antipodal nuclei do not divide again, however (fig. 14 is an exceptional case), but stain deeply and persist as such up to the time of fertilization or slightly later (figs. 15, 17, 21). At the micropylar end the two synergids are formed by the division of one nucleus, and the egg and the upper polar nucleus by the division of the other nucleus (fig. 14). Thus the mature embryo sac (fig. 15) is six-nucleate, the upper polar nucleus alone taking part in the formation of the primary endosperm nucleus.

The pollen tube penetrates through the micropyle to enter the embryo sac. During this process one of the synergids is disorganized. One sperm nucleus fuses with that of the egg and the other with the upper polar nucleus, which lies close by (fig. 16).

ENDOSPERM.—The post-fertilization development presents some interesting features. Figure 16 shows a fertilized embryo sac. The primary endosperm nucleus, which is diploid, moves to the center of the embryo sac (fig. 17) and divides (fig. 18) to form two nuclei of which the micropylar moves toward the egg and may divide to form two to four nuclei; the chalazal one gives rise to a larger number, usually seven or eight (figs. 19–23).[1] Figure 23 represents a "plate" of eight free endosperm nuclei in an embryo sac which is cut transversely. The highest number of endosperm nuclei previously recorded in the family is four in *Cypripedium* (2).

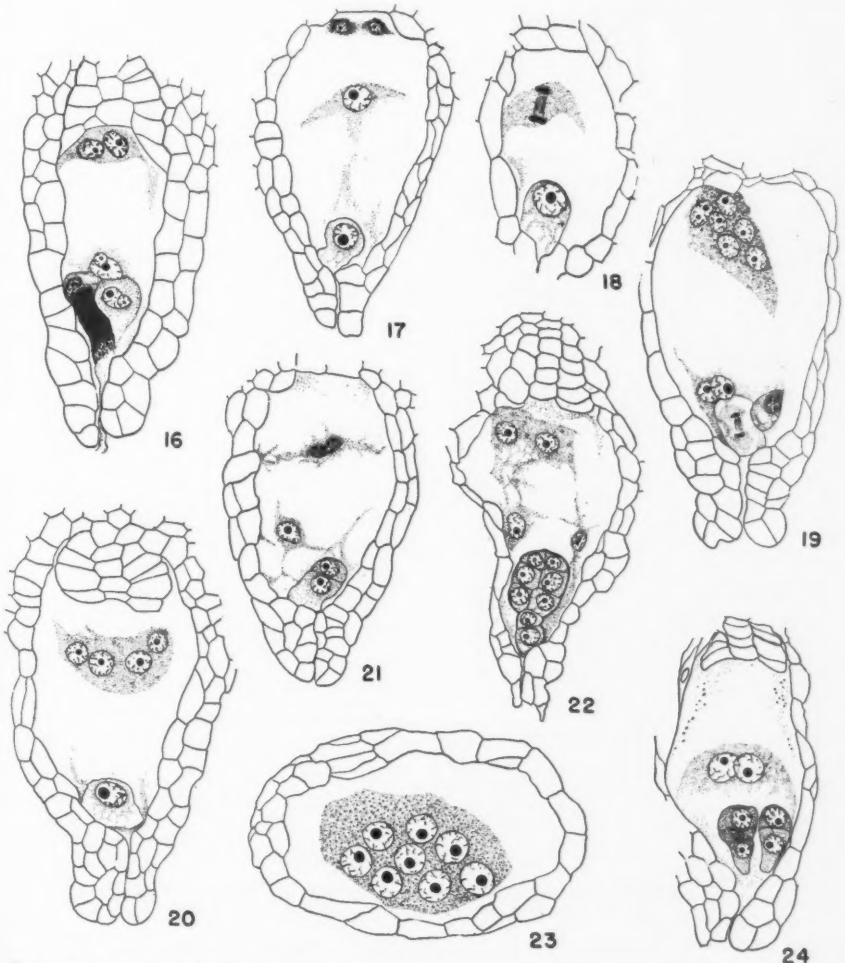
Normally the primary endosperm nucleus in *Vanilla* divides to form eight to ten free endosperm nuclei before the division of the zygote. In a few cases endosperm formation was arrested after the first or second division; figure 21 represents an instance in which even the

[1] All these figures are camera lucida drawings of single sections; only such endosperm nuclei as were visible in the particular section have been represented in a figure. However, about ten endosperm nuclei could be counted by examining the adjacent serial sections of these embryo sacs.

fusion of the two nuclei (upper polar nucleus and second sperm nucleus) is not complete, although the embryo has already attained the two-celled stage.

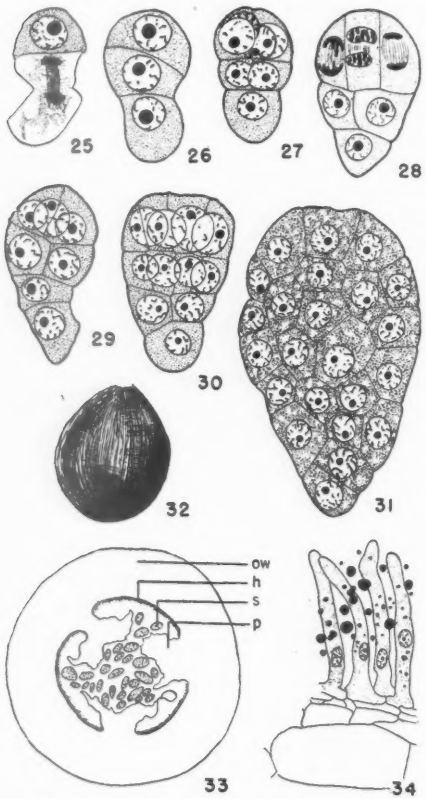
As in other orchids, the endosperm is soon used up by the growing embryo.

EMBRYO.—The first division of the zygote is transverse (fig. 21) and usually occurs after four or five endosperm nuclei are formed. By a further division of the micropylar cell (fig. 25) a proembryo of three cells is organized (fig. 26), as in the majority of orchids. The first two walls in the terminal cell are



FIGS. 16–24.—Fig. 16, double fertilization. ×600. Fig. 17, zygote, primary endosperm nucleus, and two antipodal nuclei. ×450. Fig. 18, first division of primary endosperm nucleus. ×450. Fig. 19, division of zygote; endosperm nuclei. ×450. Fig. 20, zygote and four endosperm nuclei, ×450. Fig. 21, two-celled proembryo and delayed fusion of second male nucleus with upper polar nucleus. ×450. Fig. 22, young embryo. ×450. Fig. 23, "plate" of eight endosperm nuclei (drawn from embryo sac cut transversely). ×600. Fig. 24, two proembryos lying side by side in embryo sac. ×450.

vertical and at right angles to each other, resulting in a group of four cells (fig. 27), each of which divides transversely (figs. 28, 30). Subsequent divisions are very irregular, resulting in an ovoid mass of cells (fig. 31). In embryos of mature seeds



FIGS. 25–34.—Fig. 25, basal cell of two-celled embryo in division. ×340. Fig. 26, three-celled proembryo. ×340. Figs. 27–31, subsequent stages in development of embryo. ×385. Fig. 32, mature seed; note opaque and smooth surface. ×105. Fig. 33, transverse section of fruit, showing placentae and development of unicellular secretory hairs from innermost layer of ovary. ×70. Fig. 34, secretory hairs enlarged. ×170.

there is always a well-marked depression at the chalazal end (figs. 39, 40).

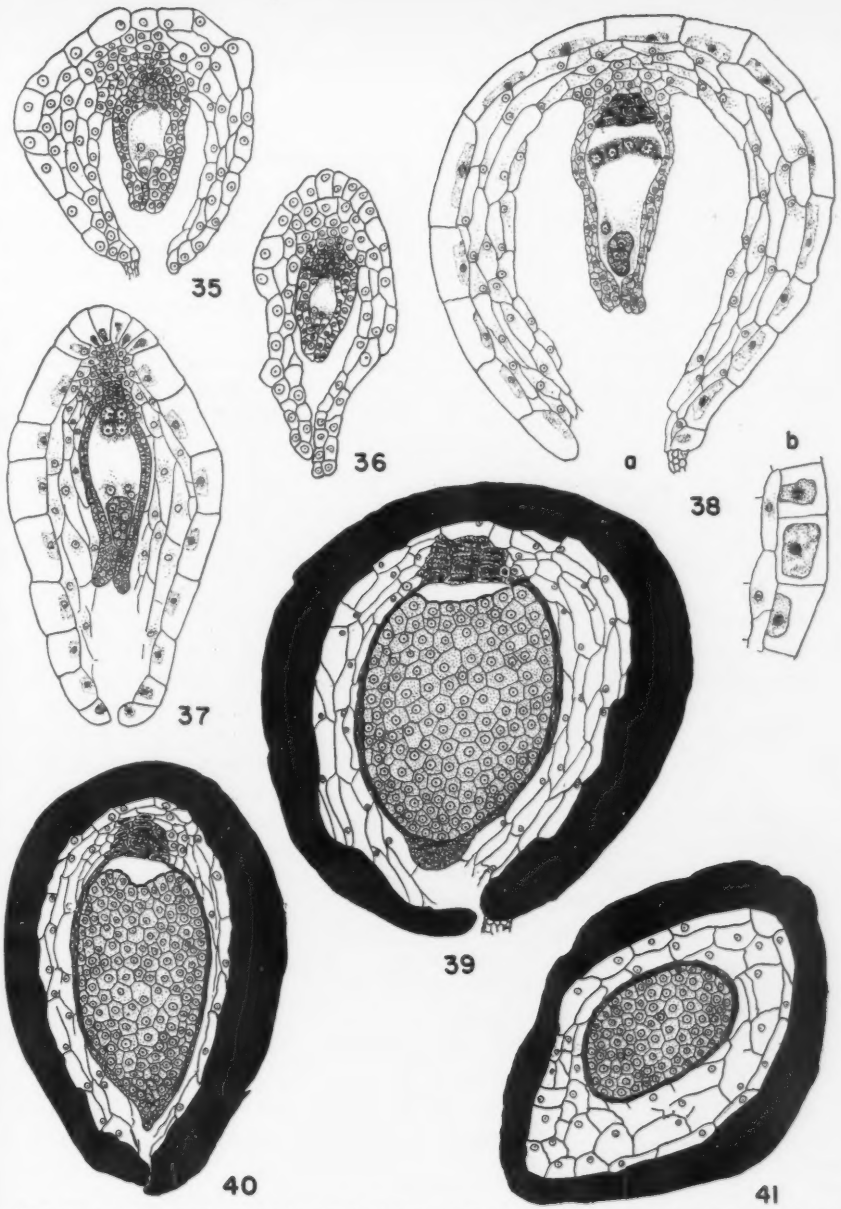
One ovule showed two embryos in the same embryo sac (fig. 24). Although the exact mode of origin of the additional embryo could not be determined, their position suggests that the three-celled embryo is the gametic one and that the two-celled embryo is derived from a synergid.

FRUIT AND SEED.—The fruit of *V. planifolia* is often described as a "bean" on account of its characteristic shape. It seems to be the only orchid possessing a fleshy fruit. RENDLE (3) has described it as dehiscing by two irregular longitudinal splits. Under cultivation, however, the pod does not show any split or rupture even when it is left on the plant for a long time and falls to the ground of its own accord.

After fertilization, the cells of the innermost layer of the ovary wall grow out into the cavity in the form of unicellular hairs which are packed with nutritive and other materials (figs. 33, 34) and are said to secrete the vanillin. They develop from the entire inner surface of the ovary wall, excepting the placentae.

In the majority of the orchids, because of the partial or complete disorganization of the cells of the inner integument, the embryo is suspended freely inside the seed coat, which is formed only from the outermost layer of the outer integument. In *V. planifolia*, however, the situation is different. Here persist not only the inner integument but also some of the original tissue of the nucellus lying at the chalazal end. These cells, which are small and very compactly arranged, stand out in sharp contrast to the large cells of the outer integument (figs. 35–40).

The epidermis of the seed coat (i.e., the outermost layer of the outer integument) undergoes important changes during the development of the embryo. The outer and lateral walls of these cells thicken enormously (figs. 37–38) until the cell cavity may be completely ob-



FIGS. 35–41. Figs. 35, 36, median longitudinal sections of young ovule (at about time of fertilization) in two planes at right angles to each other. ×42. Figs. 37, 38*a*, same as above but at later stage with young embryo and endosperm. ×42. Fig. 38*b*, three cells of outermost layer of outer integument enlarged to show gradual obliteration of cell lumen. ×270. Figs. 39, 40, sections of mature seeds in two longitudinal planes at right angles to each other. Note persistence of all cell layers of outer integument, crushed inner integument, cup-shaped depression at chalazal end of embryo, and sclerotic and opaque layer of seed coat. ×42. Fig. 41, transverse section of mature seed. ×42.

literated. Finally, a brownish-black deposit appears in the cells and intensifies to such an extent that the entire layer becomes opaque (figs. 39–41). It is smooth and highly sclerotic, without any recognizable reticulations or pattern (fig. 32), and thus offers a complete contrast to the condition in other orchids, which have a reticulate and transparent seed coat. The only cases similar to *Vanilla* are *Apostasia*, *Adactylus*, and *Selenipedium* (1).

Summary

1. In the orchid *Vanilla planifolia* the microspores do not remain united to form compound grains, massulae, or pollinia, but lie free inside the anther. The pollen grains are shed at the two-celled stage.

2. The nucellus shows a greater development than in most other orchids, and the nucellar cells at the chalazal end persist even in the mature seed.

3. The outer integument consists of four to six layers of cells which persist in the seed. In other orchids only the outermost layer is recognizable in the seed.

4. The inner integument is also two- to three-layered and, although flattened and crushed, remains distinguishable for a long time.

5. The embryo sac arises from the chalazal cell of a row of three cells, consisting of a micropylar undivided dyad cell and two megaspores. After the four-nucleate stage, the two chalazal nuclei do not divide further, resulting in a six-nucleate embryo sac.

6. Double fertilization takes place normally, but, since there is no lower polar nucleus, the primary endosperm nucleus is diploid and not triploid.

7. As many as eight to twelve endosperm nuclei were counted in certain ovules, which is noteworthy in view of the fact that ordinarily endosperm formation is completely suppressed in the orchids.

8. One case of twin embryos was noted. One of them was gametic in origin and the other probably arose from a synergid. The embryo of the mature seed shows a terminal depression.

9. Both integuments take part in the formation of the seed coat. The outermost layer of the outer integument becomes highly sclerotic with black deposits and is opaque and smooth. This is in marked contrast to the condition in most other orchids, where there is a transparent seed coat with a reticulate surface formed only by the outermost layer of the outer integument.

I am indebted to Dr. P. Maheshwari for revising the manuscript.

Biological Laboratories
Harvard University
Cambridge, Massachusetts

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CURRENT LITERATURE

Luther Burbank: A Victim of Hero Worship. By WALTER L. HOWARD. (*Chronica Botanica* **9** [5/6]: 299–522. 1945–1946.) Waltham, Mass.: Chronica Botanica Co., 1946. $3.75.

The flow of comments and writings concerning LUTHER BURBANK and his work has notably slackened during the last decade. With the lapse of time since his death in 1926, what is said about him and his work has become more objective and less prejudiced either for or against him or his efforts. The recent volume by Dr. W. L. HOWARD states its purpose in a portion of its prologue: "With none too gentle hands I have endeavored to tear away these hindering habiliments to see what the real BURBANK looked like. And behold, a pristine figure emerges that is every inch a man of worth, a man of original ideas; a man with a definite mission in life, fully capable of standing on his own feet as a lone worker in the field of science." The author states further that he is "convinced that BURBANK possessed the talent and attributes of a conventional scientist and [that] there would have been no question about his acceptance had he been taken in hand when young and given the proper training demanded by the code. . . . . For [his] concrete accomplishments and because he was the ferment that stirred others to advance the science of breeding, an intangible achievement, to be sure, but his greatest from the viewpoint of science, he is definitely entitled to a place in the hall of fame; but, all factors duly considered, the best that I can do is to assign him a front seat among the minor prophets, a front seat because of the unusual fact that here is one prophet, at least, who was not without honor among his home people."

Dr. HOWARD has based his book on his personal contact with BURBANK, conversations with many individuals who knew him intimately, BURBANK's own writings, and a firsthand opportunity to observe and evaluate many of BURBANK's horticultural creations. Although many of the latter have proved to have very little permanent value, a number of others, including the Burbank potato and several varieties of plums, have proved of great commercial importance.

When one reads of the vigor and persistence with which BURBANK was accustomed to work and of the myriad interruptions of his time and efforts by the interested or by the merely curious, he marvels not at his occasional manifestation of irritability but at his infinite patience, tolerance, and kindliness. For him to have completely resisted the adulations showered upon him, or to have withstood the flattery from others for their own personal gain, would have marked BURBANK as superhuman. That he quite obviously was not.

Dr. HOWARD's book is deserving of reading and study as an objective evaluation of BURBANK and his work. It is of equal interest in its disclosure of the author's own mind and opinions. Coming as his remarks do, from one who has had many years of experience with men and problems in the applied and scientific phases of horticulture and agriculture, one who has had broad administrative contacts in dealing with the acts and minds of men, his comments have much more than passing interest. Much of what he has said about BURBANK and about science and its workers has needed saying for some time. Those fortunate enough to have an opportunity to read this book, especially younger scientists, will wish to keep it near at hand so that they may reread it often for its stimulating pleasure, for its clarity, and for the pointedness of its comments.—E. J. KRAUS.

Garden Lilies. By ALAN and ESTHER MACNEIL. New York: Oxford University Press, 1946. Pp. xiv + 226. $3.50.

This is a rather small handbook which has been written by experienced growers and lovers of lilies for the gardener who wants to grow these plants successfully. The book is arranged in two parts.

Part I considers such topics as the selection of suitable varieties, sites for planting them in order to bring out the best effects from size of plant and type and color of flower. Practical information on planting, on culture and fertilization, and on propagation follow. Next is a chapter dealing with lilies for pots and exhibition, with suggestions regarding size of pot and bulb, soil, moisture, and temperature requirements, and a list of varieties adapted for pot culture with brief recommendations for preparing lilies for exhibition. Natural hazards and pests are listed, with suggestions for their control or amelioration. A discussion of lily diseases and their control forms a valuable and concise practical summary of the present knowledge in this area. This part is concluded with an interesting and informative discussion of lilies through the ages.

Part II is devoted largely to a descriptive list of lilies. Although the descriptions are designed not for the botanist but for the gardener, they are nevertheless accurate, concise, and generally adequate. The list comprises nearly half the book and is a valuable feature, since it furnishes much worth-while information for all who may be interested in this lovely garden plant. A selected list of references precedes the index.—J. M. BEAL.

Forest Soils and Forest Growth. By S. A. WILDE. Waltham, Mass.: Chronica Botanica Co.; New York City: G. E. Stechert & Co., 1946. Pp. 241. Illus. $5.00.

The appearance of this book fulfils an urgent need among ecologists and foresters, especially those engaged in land management, reforestation, nursery practice, and soil conservation.

A historical and introductory chapter is followed by three chapters on genesis of soils and by one on the biological structure of the forest cover and its relation to environment. There are separate chapters on physical and chemical properties of forest soils, their organisms, forest humus, soil-forest types, forest soil survey, and soils and tree-planting. Practical applications of knowledge of forest soils are brought out in chapters on amelioration of forest soils, silvicultural cuttings in relation to soils, and productivity of forest soil and forest management. The final five chapters deal with various problems in establishment and management of forest nurseries, with special emphasis on various aspects of fertilization, watering, and control of parasitic organisms. Here WILDE contributes a very large part from his own exhaustive researches conducted over a period of several decades. The book is well illustrated with thirty-eight figures (maps, charts, and graphs) and seven plates, besides a number of clearly done sketches that lend interest and a decorative motif.

The author has rather completely reviewed the literature. His firsthand knowledge of Russian and German, besides a half-dozen other languages, makes this an unusually valuable contribution.

By training, Dr. WILDE is a forester as well as a pedologist. This rare combination gives the book a balanced view of the interdependence of silviculture, ecology, and soil science that is largely lacking in existing textbooks which deal with the topic either of silviculture or of soils but fail to integrate the two subjects properly. This valuable book is thus a "must" for every ecologist and forester. It is one of the most useful of references.—J. H. STOECKELER.

Crop Production and Environment. By R. O. WHYTE. London: Faber & Faber, Ltd., 1946. Pp. 372. Illustrated. 12*s*.

Under this general title one might reasonably expect a discussion of any aspect of applied plant science, but, obviously, one book could not cover all aspects of crop production and environment. On the basis that the physiological processes leading to growth and reproductive development are fundamental to crop production, WHYTE deals with the literature related to the initiation and development of flowers in plants as influenced by the decisive environmental factors, light and temperature.

The book is not simply a review but is a very reasonable attempt to evaluate and to correlate the literature in the fields of photoperiodism, vernalization, and phasic development. The author has selected certain critical investigations for detailed consideration and has included many excellent illustrations taken from the original papers. An extensive review of the factual data is presented, and considerable space is devoted to the various hypotheses which have been developed in relation to some of the facts. This latter inclusion is fortunate, since the obvious inadequacy of any one of the hypotheses in relation to all the available facts demonstrates the need for more critical experimentation. The similarity among some of the more recent generalizations encourages one to hope that soon additional generalizations will serve to correlate the flowering responses of most or even of all plants with changing environmental conditions.

WHYTE uses the terms "growth" and "development" in a restricted sense and defines them as follows: "Growth is the accumulation of dry matter or increase in size of a plant, development of progress towards reproduction by externally recognizable or invisible stages." The book is primarily concerned with the problems of development.

The Introduction gives an outline of the scope of the book, and the first chapter includes a historical review of the development of the studies on photoperiodism and vernalization, with some detail of the early work of KLEBS, GASSNER, and GARNER and ALLARD. There follows a chapter on the concept of phasic development, giving a concise statement of LYSENKO's work and hypothesis, together with the modifications introduced by subsequent workers. In the remaining chapters the influences of darkness, light, and temperature are discussed in relation to photoperiodism and phasic development. Extensive reference is made to the Russian literature. Most of the hypotheses which have been developed in connection with this work receive considerable attention. One brief chapter is devoted to the problem of breeding plants in relation to phasic analysis—a welcome presentation of a controversial subject. The last four chapters discuss the responses of economic crops and possible practical application of our knowledge of developmental physiology.

This book should prove to be valuable to those interested in the physiological processes leading to flowering in plants, and it should be especially invaluable to the research worker. Perhaps its greatest contribution will be in its stimulation of new research, since it summarizes the present status of our information in this field and points the way for new investigations to fill in the gaps in our knowledge.—K. C. HAMNER.